ABSTRACT

DALEY, JAMES DANIEL. Screening for Resistance to Bacterial Fruit Blotch in Watermelon Fruit (Under the direction of committee chair Dr. Todd C. Wehner).

Bacterial fruit blotch (BFB), caused by the pathogen *Acidovorax citrulli,* is a major disease affecting watermelon fruit and transplant production around the world. Bacterial fruit blotch is particularly destructive because it is a seedborne disease that presents inconspicuous foliar symptoms and can spread rapidly in transplant facilities and production fields. Serious BFB outbreaks cause major losses every year, and infested seed detection and exclusion methods represent a significant cost to the seed industry. Despite four decades of research and resistance screening, there are no commercial cultivars with BFB resistance.

While there have been many studies of foliar resistance, studies of resistance in the fruit have been largely neglected. Only a few minor fruit screening studies have been reported and screening methods have not yet been well established. From 2015 to 2017, we evaluated several fruit inoculation methods using inoculum concentrations of 10^3 to 10^8 CFU/ml for use in large-scale field resistance screenings. The following factors have been studied: (1) immature fruit spray inoculation under wounded vs. unwounded and bagged vs. unbagged conditions; and (2) mature fruit injection inoculations. ‘Charleston Gray’, ‘Mickylee’, and ‘Crimson Sweet’ were evaluated in 2015 and 2016, with no significant differences in disease severity observed among cultivars. ‘Sugar Baby’ and ‘Charleston Gray’ were evaluated in 2017. In spray inoculation tests, ‘Sugar Baby’ had significantly lower disease severity than ‘Charleston Gray’ but was significantly more susceptible than ‘Charleston Gray’ using the injection-inoculation method. These results suggest that fruit resistance may be based on fruit surface mechanisms. In general, all inoculum concentrations produced similar disease severity for spray inoculations and only the lowest concentration, 10^3 CFU/ml, resulted in significantly lower disease severity in injection

inoculations. In 2015 and 2016, bagging and wounding immature fruit led to a high rate of fruit abortion and were not evaluated in 2017. Among the treatments evaluated, the simple spray inoculation method on 1- to 2-week-old fruit produced the most consistent results and was the most amenable method for large-scale screenings.

During methods development, we also screened 1433 *Citrullus* spp. accessions and 19 watermelon cultivars for BFB fruit resistance under field conditions. We identified resistant cultigens and estimated broad-sense heritability for resistance. During the three-year study, broad-sense heritability (H^2) was estimated to be 0.28. However, follow-on screenings under similar conditions could attain much higher H^2 estimates with different resource allocations. Based on variability estimates from our screening study, 15 resource allocations were simulated and H^2 estimated. In one scenario that simulated two years with eight blocks per year and four replications per cultigen, H^2 was predicted to be 0.66. Overall, year was not a large source of variation, and scenarios that maximized blocks within year and replication had the highest estimated H^2.

Of the resistant lines observed in the screening blocks, a subset of 40 were chosen for retesting in 2016 and 2017. Among these lines, PI 494819 (*C. lanatus*), PI 596659 (*C. amarus*), PI 596670 (*C. amarus*), PI 490384 (*C. mucosospermus*), and PI 596656 (*C. amarus*) consistently had the lowest disease severity and lowest advanced symptom incidence. These PI accessions could be used for cultivar development and studies of fruit resistance mechanisms.

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Screening for Resistance to Bacterial Fruit Blotch in Watermelon Fruit

By
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APPROVED BY:

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BIOGRAPHY

I was born in 1982 and spent most of my childhood living on the foothills of the Rocky Mountains in Utah. The hills and mountains were my year-round playground, nurturing the fledgling natural scientist within. Sensing an early passion and curiosity for nature, I pursued biology in my studies for the rest of my academic career.

After graduating from high school in 2000, I interrupted my studies to serve for two years as a missionary in Japan for The Church of Jesus Christ of Latter Day Saints where I taught the gospel and partook of the extreme kindness of the Japanese people. From that experience my faith increased, and I developed many lifelong friends. Shortly after returning from Japan, I enlisted in the Army National Guard in 2004 and commissioned as an officer in 2008. The Army taught many important leadership skills and provided a multitude of life experiences. I consider it an honor to have had the opportunity to serve my country.

During my undergraduate experience at Brigham Young University, I attended a seminar in which a plant breeder spoke about the rewards of plant breeding. At the time I was unsure of the direction I wanted to take but after hearing that seminar I felt that plant breeding would be an extremely satisfying career. After finishing my B.S. in biotechnology in 2008, and while on a deployment in Afghanistan in 2010, I discovered Dr. Todd Wehner's cucurbit breeding program online and thought that it would be a great opportunity. It wouldn't be until 2014 that I would meet Dr. Wehner at the USDA Vegetable Laboratory in Charleston, SC and would be admitted into his program.

The greatest thing that has happened to me was meeting my wife Shawna in late 2006 in an introduction to soil science course at Brigham Young University. We dated the following

year and were married on December 29, 2007. In 2014, she gave birth to Gretchen Leigh and, in 2017, to Amelie Jane. My family continues to be a boundless source of joy.

After returning from Afghanistan in 2011, I quit my job as a medical pathology technician at ARUP in Salt Lake City, and my wife and I both started our Master's studies in plant and environmental science at Clemson University. We had an extraordinary experience conducting our Master's research at the USDA Vegetable Laboratory in Charleston, SC under the direction of Dr. Pat Wechter and Dr. Richard Hassell. I learned many invaluable lessons from them as a budding professional scientist. I also enjoyed the southern climate and running on the farm with our dog Rudy.

During my time at Clemson and the USDA station in Charleston, I continued to see that a career as a commercial plant breeder aligned with my interests. In 2014, I was accepted into the horticulture Ph.D. program at NC State University, and my 2010 hope of being part of the NCSU cucurbit breeding program was realized. Our family grew and we made extraordinary friends in Raleigh. At NCSU, I learned the practicalities of field breeding from Dr. Wehner and expanded my statistical and genetic skills through professional associations.

The next chapter of my journey is in Davis, California as I have joined H.M. Clause as the pumpkin breeder squash prebreeder. While few things have been easy, I have been richly blessed with opportunities and a wonderful family. I have no doubt that continued great challenges and fulfilling experiences await.

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Chapter 1

A Review of Bacterial Fruit Blotch of Watermelon

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1 INTRODUCTION

Watermelon *Citrullus lanatus* [Thunb.] Matsum. & Nakai is a valuable crop grown on over 8.5 million acres around the world in 2012 (Food and Agriculture Organization of the United Nations, 2014). In 2015, the US harvested 47,125 ha of watermelon at a total value of over $488 million dollars (USDA National Agricultural Statistics Service, 2017). Watermelon is part of the Cucurbitaceae family which includes many important crops such as melon (*Cucumis melo* L.), squash (*Cucurbita moschata* L.), and cucumber (*Cucumis sativus* L.).

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* is a major seed-borne disease that affects watermelon (*Citrullus lanatus*) seedling and fruit production around the world (Latin and Hopkins, 1995, Schaad et al., 2003, Bahar and Burdman, 2010, Tian et al., 2016, Carvalho et al., 2013, Palkovics et al., 2008). The BFB causal agent was first isolated in 1965 at the Regional Plant Introduction Station, Experiment, GA from two watermelon plant introductions (PIs) that presented water-soaked lesions on their leaves (Webb and Goth, 1965). It was first classified in 1978 as *Pseudomonas pseudoalcaligenes* subsp. *citrulli* (Schaad et al., 1978), in 1992 as *Acidovorax avenae* subsp. *citrulli* (Willems et al., 1992), and in 2008 it was reclassified to its current nomenclature *Acidovorax citrulli* (Schaad et al., 2008). Initially, *A. citrulli* was considered a mere scientific interest (Latin and Hopkins, 1995); however, that changed with the first loss report of confirmed BFB in commercial watermelon fields in the Mariana Islands in 1987 where it was coined “fruit blotch” (Wall and Santos, 1988). In 1989, it was found in watermelon fields in the United States (Hopkins, 1989); the initial outbreaks in South Carolina

and Florida caused watermelon losses nearing 80% (Hopkins et al., 1993). BFB has since spread to most watermelon producing areas of the United States (Somodi et al., 1991, Wall et al., 1990, Hamm et al., 1997, Evans and Mulrooney, 1991, Black et al., 1994), and, in 1994, infections occurred in thousands of acres of watermelon across 10 states (Hopkins and Thompson, 2002b). Outbreaks of BFB can lead to a complete loss of the primarily infected field and serious damage, 5-50%, to nearby by fields through secondary outbreaks (Latin and Hopkins, 1995). Rampant infections in the mid-1990's incited a period of "high stakes" litigation of seeds companies by growers which caused a near shutdown of watermelon production in the US in 1995 (Latin and Hopkins, 1995). Because *A. citrulli* is a worldwide threat, contaminated plant import restrictions exist in the USA, China, and Europe (Tian et al., 2016). In 1996, *A. citrulli* was reported in honeydew melon fields in Texas (Isakeit et al., 1997), and although most reports and studies focus on watermelon infection, BFB also poses a significant threat to melon (*Cucumis melo*) (O'Brien and Martin, 1999) and pumpkin production (Langston Jr et al., 1999) and is able to cause foliar symptoms and infest seed of all major cucurbits and cause fruit symptoms on most when inoculated (Hopkins and Thompson, 2002a). BFB has proven to be highly dangerous to the watermelon industry for five primary reasons: the high genetic diversity of *A. Citrulli* has negated prior identified resistance (Bahar and Burdman, 2010); the often subtle symptoms make elimination from production facilities challenging (Hopkins et al., 2009); sporadic infection spreads rapidly under humid conditions and can lead to complete losses (Latin and Hopkins, 1995); resistance is dependent on the plant developmental stage (Bahar et al., 2009b, Johnson et al., 2011); and seed infestation leads to wide dispersal of the pathogen (Rane and Latin, 1992).

The unprecedented and worldwide *A. citrulli* threat has focused the industry and researchers toward the common goal of controlling BFB (Latin and Hopkins, 1995). Bacterial

fruit blotch is a watermelon top research priority (King and Davis, 2005) and was more recently increased to third in research priority just behind fusarium wilt *Fusarium oxysporum* f. sp. *niveum* and gummy stem blight resistance *Didymella bryoniae* (Kousik et al., 2016). Because of its insidious nature of the infection and dispersal, BFB presents a prominent danger to watermelon production worldwide.

Currently, the most effective control measure is the careful exclusion of infected plants at seedling production facilities and, ultimately, fields (Hopkins and Thompson, 2002b, Hopkins et al., 1996). To that end, research has focused on enhancing BFB detection through molecular techniques (Walcott and Gitaitis, 2000, Walcott et al., 2006, Bahar et al., 2008, Ha et al., 2009). Moderate chemical control can be achieved in the field through the repeated application of copper containing products during early fruit development (Hopkins, 1991, Hopkins et al., 2009), but copper insensitivity has already been reported in some *A. citrulli* strains (Walcott et al., 2004). Additionally, some promising biocontrol methods have also been investigated (Johnson et al., 2011). However, despite detection efforts and control measures, scattered BFB outbreaks continue to cause significant damage to watermelon production (Hopkins et al., 2009).

Although there have been many BFB resistance screenings in watermelon (Hopkins et al., 1993, Hopkins and Thompson, 2002a, Ma and Wehner, 2015, Carvalho et al., 2013, Goth and Webb, 1981, Sowell and Schaad, 1979), there are no resistant commercial watermelon lines available (Johnson et al., 2011). Furthermore, beyond evidence for polygenic genetic control (Hopkins and Levi, 2008), BFB resistance inheritance is largely unknown. BFB resistance would be a highly valuable for mitigating the effects of BFB and could be become an important integrated pest management (IPM) program.

2 *ACIDOVORAX CITRULLI*

2.1 Characteristics and Lifecycle

Acidovorax citrulli bacterium are gram-negative rods that average 0.5µm by 1.7µm in size, possess a single polar flagellum measuring 5.0µm (Schaad et al., 1978), and grow optimally at 27°C to 30°C (Burdman and Walcott, 2012). *A. citrulli* can infect all growth stages of the watermelon plant: seeds, seedlings, foliage, flowers, and fruit (Latin and Hopkins, 1995). Infested seed are sown in humid transplant houses with overhead irrigation, ideal circumstances for bacterial growth and splash propagation, resulting in rapid infection of nearby plants (Latin and Hopkins, 1995). Infected seedlings become serious sources of inoculum in the field, resulting in rapid dispersal of the pathogen from leaf to leaf (Latin and Hopkins, 1995). Seedling symptoms are water-soaked, brown lesions on the cotyledons and hypocotyl which often leads to plant death (Latin and Hopkins, 1995). Leaf lesions are often discreet, "small, dark brown and somewhat angular" (Latin and Hopkins, 1995) and tend to present along the major leaf veins (Hopkins et al., 1993). Infected plants are generally not defoliated, but rather leaf tissue becomes a reservoir for *A. citrulli* that later spread to developing fruit (Bahar et al., 2009b, Latin and Hopkins, 1995, Hopkins and Thompson, 2002b, Frankle et al., 1993). Rane and Latin (1992) found that while the pathogen could be isolated from blotch margins on the fruit, it was not found in the peduncle and stems, suggesting that fruit infection does not appear to occur systemically through the vine. However, researchers studying group I strain mobility presented evidence suggesting that A. *citrulli* is able to infect and migrate in the xylem of seedlings (Bahar et al., 2009a). The degree to which vascular infection may contribute to fruit and seed infection has not been thoroughly investigated.

Watermelon fruit are most vulnerable to *A. citrulli* bacterium entering through stomata during the first two weeks post anthesis and are susceptible up to five weeks post anthesis, prior to stomatal plugging by waxy cuticle (Frankle et al., 1993). Fruit symptoms quickly progress from inconspicuous water-soaked lesions with irregular margins to expanded dark-green lesions that fissure, causing massive internal infection by secondary organisms, which leads fruit collapse (Latin and Hopkins, 1995, Hopkins et al., 1993). As *A. citrulli* Seeds are internally and externally infested with *A. citrulli* bacterium (Rane and Latin, 1992), completing the disease cycle. Infestation can also occur in symptomatic fruit or asymptomatic fruit exposed to the pathogen (Hopkins et al., 1996, Carvalho et al., 2013) or through a blossom infestation pathway (Walcott et al., 2003). Walcott et al. (2003) suggested that the presence of *A. citrulli* in the fruit pulp without the characteristic fruit rot may provide evidence that *A. citrulli* itself is not the causal agent of rot symptoms, but rather invasion by opportunistic microbes. Seed contamination does not appear to be affected by storage under dry conditions over 12 months and would likely not be eliminated by longer-term storage (Hopkins et al., 1996). Indeed, we have isolated *A. citrulli* from four-year-old seed stored under cool, dry conditions. Insidious seed infestation continues to spread the pathogen around the world, representing a serious threat to the watermelon industry.

2.2 Diversity and Pathogenicity

In 2000, based on DNA fingerprinting and gas chromatographer-fatty acid methyl ester profiles, *A. citrulli* strains were further subdivided into to two groups: I and II (Walcott et al., 2000, Walcott et al., 2004) and, in 2014, a third *A citrulli* group was identified based on rep-PCR profiles, box-PCR, type three secretion system (TTSS) effector sequences, and virulence difference (Eckshtain-Levi et al., 2014). It is generally accepted that group I strains are

moderately aggressive on watermelon and non-watermelon cucurbits; group II strains are highly aggressive on watermelon and show low aggressiveness on non-watermelon cucurbits (Walcott et al., 2004, Eckshtain-Levi et al., 2016, Zivanovic and Walcott, 2017, Burdman et al., 2005); and group III isolates were found to be weakly virulent, relative to group I and II isolates, on watermelon and non-watermelon cucurbits (Eckshtain-Levi et al., 2014). In contrast to the group I and group II aggressiveness on watermelon paradigm given by Walcott et al. (2004), Eckshtain-Levi et al. (2014) found no significant severity difference on watermelon for either group I and group II; however, the overall trends reported by Eckshtain-Levi et al. (2014) are comparable to other virulence findings (Burdman et al., 2005, Walcott et al., 2004). Overall, high variability for disease severity within groups (Eckshtain-Levi et al., 2014) make it so that disease severity rankings could also vary depending on the strains compared.

Comparative genomic analysis has revealed different pathogenicity mechanisms and group I and group II strain divergence. In a landmark study, Eckshtain-Levi et al. (2016) sequenced a group I strain, M6, and compared it to a group II strain (AAC00-1) sequence (GenBank accession NC_008752) provided by the Joint Genome Institute and identified interesting differences: the M6 genome, 4.85 Mb, is smaller than the AAC00-1 genome, 5.35 Mb; M6 has 132 unique and 4,245 shared open reading frames (ORFs) and AAC00-1 has 532 unique ORFs and 4,405 shared ORFs; and the unique genes in AAC00-1 are clustered in eight regions spread throughout the genome. When other group I and group II strains were surveyed using markers for the AAC00-1 unique gene clusters, the clusters were shown to be highly conserved among group II strains and rare in group I strains (Eckshtain-Levi et al., 2016). Based on the group differences, Eckshtain-Levi et al. (2016) speculated that early group I strains gradually accumulated these regions in response to adaptation and became the group II strains; as

further evidence for group I origination hypothesis, all of the pre-1987 outbreak isolates *A. citrulli* were group I. Beyond evolutionary implications, these genomic differences undoubtedly contribute to ecological adaptations and host preferences.

The *A. citrulli* genomes have been shown that *A. citrulli* contains putative hypersensitive response and pathogenicity (Hrp) genes and type three secretion system (TTSS) effectors (Bahar and Burdman, 2010, Eckshtain-Levi et al., 2014, Eckshtain-Levi et al., 2016, Bahar et al., 2009a). When Eckshtain-Levi et al. (2014) compared 11 TTSS gene sequences across 22 *A. citrulli* strains they found that TTSS gene variations differentiated the strains into previously described groups I and II, as well as identified the third group. These TTSS effector differences likely reflect host selection pressure differences and contribute to group host preferences (Eckshtain-Levi et al., 2014). Based on these effectors, Zivanovic and Walcott (2017) developed group-specific primers for simple and rapid isolate differentiation, which had been previously done using more labor intensive genetic and biochemical assays (Burdman et al., 2005, Feng et al., 2009b, Walcott et al., 2004). Mutation studies have shown that mutant strains with defective effectors lack pathogenicity on watermelon or melon. For example, Bahar et al. (2009a) demonstrated that mutant strains with a defective TTSS gene, *hrcV*, lacked pathogenicity on melon and watermelon and failed to cause a hypersensitivity response on nonhost plants. In an interesting study exploring biocontrol, Johnson et al. (2011) created nonpathogenic *A. citrulli* by creating a TTSS, *hrcC*, mutant AAC00-1. More recently, Yan et al. (2017) showed that a mutant M6 strain for the *hrcV* pathogenicity gene failed to cause symptoms on immature melon fruit. The number of predicted TTSS effectors is probably underestimated (Eckshtain-Levi et al., 2014), and future studies will continue to unravel the *A. citrulli*-host interaction. Further

understanding the genetics of pathogenicity will improve control methods and contribute to genetic studies of resistance (Bahar et al., 2009b).

It is generally accepted group I strains are associated with non-watermelon cucurbits and group II strains are associated with watermelons (Walcott et al., 2004). Indeed, isolates from the United States, Israel, Taiwan, China, and Australia had an overall strong host preference by group: 82% of group I isolates were found on melon (27/33) and over 93% of group II isolates (28/30) were found on watermelon (Walcott et al., 2004). However, this preference inexplicably was found not to be the case among isolates from China where all 14 strains isolated from watermelon typed as group I (Feng et al., 2009b) and in Brazil where 66/67 isolates were group I, regardless of host (Silva et al., 2016). Moreover, Silva et al. (2016) reported a similar group I predominance in China and a group II predominance in Georgia, USA, which was unexpected because global propagation via infested seed should limit group clustering. While the specific genes involved remain unknown, Silva et al., (2016) demonstrated that at high temperatures, 40°C and 41°C, group I strain growth was suppressed relative to group II strain growth, suggesting that ecological adaptation that could partially explain the geographic clustering. Evidence for different ecological adaptations of group I and group II isolates reported by Silva et al. (2016) are analogous to the host-driven TTSS stain differences between the three *A. citrulli* groups reported by Eckshtain-Levi et al. (2014). The genomic differences between group I and group II strains (Eckshtain-Levi et al., 2016) likely provide the *A. citrulli* groups competitive adaptations, such as the group II heat tolerance (Silva et al., 2016) and effector arsenals (Eckshtain-Levi et al., 2014), that explain *A. citrulli* geographic patterns and host preferences, though more research is required.

3 CONTROLLING BFB IN WATERMELON

3.1 Chemical and Biological Control

Because resistant commercial lines do not exist (Johnson et al., 2011), alternative chemical and biological control methods have been explored. In order eliminate *A. citrulli* from infested seed a variety of seed treatments have been investigated: sodium hypochlorite, hydrochloric acid and fermentation (Hopkins et al., 1996); streptomycin and sodium hypochlorite (Sowell and Schaad, 1979); heat treatments (Wall, 1989); peroxyacetic acid (Hopkins et al., 2003); sodium hypochlorite, peroxyacetic acid, and acidified cupric acetate (Feng et al., 2009a); and sodium hypochlorite, 50°C water, and hydrochloric acid (Rane and Latin, 1992); and streptomycin sulfate and hydrochloric acid. Seed treatments are often ineffective because *A. citrulli* under the seed coat are difficult to access (Rane and Latin, 1992, Hopkins and Thompson, 2002a). However, results from seed treatment assays are promising, but, in some cases, there can be marked decreased germination post treatment (Feng et al., 2009a). Another control strategy is to limit the spread of the pathogen during transplant production by overhead irrigation with copper-based bactericides and peroxyacetic acid (Hopkins, 1995, Hopkins et al., 2009). Greenhouse BFB control is more effective when bactericides are combined with acibenzolar-S-methyl, a fungicide that induces systemic resistance (Hopkins et al., 2009). In the field, copper-based bactericides can also be used to provide some BFB control (Somodi et al., 1991, Hopkins and Thompson, 2002b); its use in combination with systemic resistance inducing acibenzolar-S-methyl has not been explored. Although copper-containing bactericides do provide some control of BFB in the field and greenhouse, widespread copper application would increase the risk of copper-resistance

(Hopkins, 1995), and, indeed, group 1 strains resistant to copper were observed *in vitro* (Walcott et al., 2004).

A variety of biological control methods have been shown to reduce, but not eliminate, seed infestation, seedling transmission, or leaf colonization by competing with virulent *A. citrulli* bacterium (Johnson et al., 2011, Fessehaie and Walcott, 2005) or by producing antimicrobial substances (Adhikari et al., 2017, Fan et al., 2017, Fessehaie and Walcott, 2005). In the earliest demonstrated biocontrol, Fessehaie and Walcott (2005) identified two bacterial strains, *Acidovorax avenae* subsp. *avenae* (AAA 99-2) and *Pseudomonas fluorescens* (A506), that inhibited *A. citrulli.* Interestingly, these two strains likely utilize different inhibition mechanisms: AAA 99-2, may preemptively utilize resources and out compete *A. citrulli*; A506 produces iron-containing siderophores that have antimicrobial properties (Fessehaie and Walcott, 2005). Because *Acidovorax avenae* subsp. *avenae* is pathogenic to maize, making it unusable in commercial settings, Fessehaie and Walcott (2005) suggested that a mutagenized nonpathogenic strain could be used. In 2011, Johnson and Walcott found that a nonpathogenic *A. citrulli* TTSS mutant AAC00-1, *hrcC*, delayed symptom onset in infested seedlings and reduced BFB seedling transmission by 81.8% compared to a 77.4% reduction using AAA 99-2. Although the mutant, *hrcC,* lost pathogenicity, it was still able to colonize at normal rates and probably compete with wild-type *A. citrulli* for nutrients (Johnson et al., 2011). In 2017, Adhikari et al. identified two bacterial strains, *Paenibacillus polymyxa* (SN-22) and *Sinomonas atrocyanae* (NSB-27) that inhibited *A. citrulli* growth *in vitro*, decreased disease severity on watermelon seedlings and, interestingly, increased chlorophyll, plant height, fruit weight, and dry weight relative to controls. Similar to speculation by Fessehaie and Walcott (2005), Adhikari et al. (2017) also attributed some of SN-22 and NSB-27 antagonistic properties to siderophores production, though

confirming research are still required. In another study, Fan et al. (2017) demonstrated that surfactin, as powerful surfactant, produced by a strain of *Bacillus subtilis* inhibits *A. citrulli in vitro* and reduces *A. citrulli* growth on melon seedlings. In order to confirm the surfactin-mediated inhibition, Yan et al. (2017) found that a surfactin mutant strain of *B. subtilis* failed to reduce *A. citrulli* growth. The four studies conducted on *A. citrulli* biocontrol have identified six strains that either compete for resources or produce antibacterial compounds. To date, no studies have examined the synergistic effect on *A. citrulli* growth from combining these strains. Although promising, currently, biocontrol agents do not provide perfect protection to BFB, but could be part of integrated pest management program (Johnson et al., 2011).

3.2 Detection and Exclusion

The exclusion of *A. citrulli* from detection and elimination of disease plants from seed and transplant production and fields is the most effective control measure for controlling BFB (Hopkins and Levi, 2008, Bahar et al., 2008). The USDA National Seed Health System gives guidelines and procedures for three detection methods: grow-out assay, seedling PCR, and ISHI Method (seed-based detection). The standard detection technique is the seedling grow-out assay where 10,000-30,000 seeds per lot are grown for three weeks and compared to positive controls to determine infection (USDA National Seed Health System, 2017). Indeed, early use of grow-out tests by seed companies in the mid-1990's significantly eliminated the incidence of infested seed being sold (O'Brien and Martin, 1999). The detection of even a small number of infested seedlings results in the loss of an entire seed lot in order to prevent BFB from spreading to growers' fields (Bahar et al., 2009b). Furthermore, with the increased demand for more expensive triploid seed (Maynard and Elmstrom, 1992), the grow-out assay costs have increased. As an alternative to the slow and wasteful grow-out assays, research on optimizing PCR-based

detection has involved the selection of high-stringency primers and techniques to reduce interfering molecules, allowing reliable detection to as low as 0.02% infestation of test seed lots (Bahar et al., 2008). Polymerase chain reaction-based methods provide speed, avoiding the three-week observation period, sensitivity and specificity (Bahar et al., 2008, Walcott et al., 2006, Ha et al., 2009), but can often fail to distinguish live and dead *A. citrulli* cells (Tian et al., 2016). For example, it's conceivable that PCR methods used on infested seed lots that have undergone seed treatments could detect DNA from dead *A. citrulli* cells and give a false positive and the loss of the seed lot. In order to mitigate these false positives from dead *A. citrulli* cells, Tian et al. (2016) developed a selective real-time PCR method that allows detection of 10^3CFU/ml viable cells from among dead cells using DNA-intercalculating propidium monoazide that decreases amplification of unprotected dead cell DNA by crosslinking (Nocker et al., 2006). Seed exclusion methods are a valuable part of a multipronged approach to limiting BFB outbreaks, and advancements in detection methods have made *A. citrulli* infested seed exclusion increasingly efficient.

3.3 BFB Resistance

BFB resistance would be a highly valuable in reducing current costly preventative measures and could be become an important part of integrated pest management (IPM). Beginning before the major US outbreaks and continuing until 2015, there have been many laboratory, greenhouse, and field screenings evaluating foliar resistance in watermelon (Hopkins et al., 1993, Hopkins and Thompson, 2002b, Carvalho et al., 2013, Ma and Wehner, 2015, Hopkins and Levi, 2008, Sowell and Schaad, 1979) and melon (Bahar et al., 2009b, Wechter et al., 2011). These resistance studies are summarized in Appendix A among other historical inoculation method applications. While the identification of BFB resistance in developed

cultivars would have the definite advantage of being associated with desirable traits, Hopkins et al. (1993) concluded, after an extensive cultivar resistance screening, that none of the tested cultivars were immune and that some of the more resistant cultivars tested were among the highest commercial field losses in Florida (70-80%). Because commercial cultivars are unlikely candidates for bacterial fruit blotch resistance (Hopkins et al., 1993), Hopkins and Thompson (2002) conducted a wide germplasm screen of 1,344 *Citrullus* spp. and *Praecitrulllus fistulosus* PI seedlings, in which two PIs were identified as strongly resistant. These two PIs were used in a backcrossing program designed to introgress resistance into Crimson Sweet (Hopkins and Levi, 2008). In the first large-scale field screening for resistance in mature leaves, Shen and Wehner (2014) tested 1699 *Citrullus* spp. cultigens over three years and identified 23 resistant lines. Unfortunately, as has been seen other lines, the most resistance cultigens have poor horticultural traits that, assuming polygenic inheritance, will require extensive backcrossing. Moreover, most of the lines tend to be *Citrullus amarus* instead of *Citrullus lanatus.* Due to the recent nature of the study it remains to be see if resistance will be introgressed into commercial varieties. Despite the intensive efforts by many researchers, there are no resistant commercial watermelon lines available (Johnson et al., 2011).

While the majority of majority of resistance has focused on watermelon, there are other of screenings that have focused on *Cucumis melo* L. (Wechter et al., 2011, Bahar et al., 2009b) . Even though identified resistant melon cultigens are of little use to watermelon breeders, the similar pathology, insight gained concerning resistance mechanisms, and resistance screening methodologies have direct application to studies of BFB of watermelon. Bahar et al. (2009b), after exposing melon seeds to inoculum, found that disease severity was associated with pathogen adherence to the seed coat. Susceptible melon lines were found to have a two to six

times as many CFU/seed than resistant lines and group 1 strains had significantly higher adherence than group 2 strains (Bahar et al., 2009b). The correlation of bacterial quantity and susceptibility suggests that the resistance may be partially explained as avoidance. This type of mechanism may also exist in watermelon. In a different experiment, Wechter et al. (2011) introduced a novel method of vacuum-infusion of *A. citrulli* into melon seeds, in an effort to simulate seed infestation. Similarly, in watermelon, soaking uninfected watermelon seeds produced internally and externally infested seeds, mimicking natural infestation and disease symptoms (Rane and Latin, 1992). Rane and Latin (1992) suggested that infiltration of seeds exposed to inoculum likely occurs through the movement through small holes in the hilum. This concurs with the presence of air bubbles at the hilum region of melon seed when vacuum is applied as per the method used in melon by Wechter et al. (2011), and suggests that a vacuum assay would be an effective seed infestation method for watermelon. Although BFB research has heavily focused on watermelon, particularly during the 1990's and early 2000's, understanding BFB control measures and resistance on alternate hosts, such as melon, will be valuable for watermelon researchers.

Disease resistance appears to be dependent on both environmental condition, developmental stage (Carvalho et al., 2013, Bahar et al., 2009b, Hopkins and Thompson, 2002b), and *A. citrulli* isolate genetic diversity and aggressiveness (Walcott et al., 2000, Johnson et al., 2011, Bahar and Burdman, 2010). For example, Hopkins et al. (1993) attributed their failure to reproduce prior resistance in 'Congo,' PI 295843, PI 299378 (Sowell and Schaad, 1979) and 'Garrison' (Goth and Webb, 1981) to strain differences or environmental effects. In melon, Carvalho et al. (2013) found that certain lines were resistant when inoculated before flowering and susceptible after flowering. In another study, seedling and fruiting stages appeared to the

most susceptible to BFB, whereas infection of other stages of development can be difficult to detect, leading to the appearance of high resistance (Bahar et al., 2009b). However, even when inoculations are conducted early in development, Wechter et al. (2011) reported that some PIs that were resistant in vacuum-infusion assays were highly susceptible in seedling spray inoculation assays. In order to mitigate developmental and strain-host interactions that have led to repeatability issues with early identified resistance, researchers rely on two strategies to identify broad resistance: (1) expose the host to a range of pathogen diversity by using multiple group I and group II strains; (2) conduct resistance screening over multiple developmental stages and environments. Ideally, greenhouse resistance would ultimately be confirmed in field studies. Bacterial fruit blotch resistance screenings must be considered in light of the environmental conditions, developmental stage, and the particular *A. citrulli* strains used.

Resistance in the fruit would allow growers to produce marketable fruit, in spite of BFB presence in the field, and because fruit resistant watermelons have lower incidence of seed infestation (Carvalho et al., 2013), seed producers may also benefit. In their extensive PI screening Hopkins and Thompson (2002) did not observe BFB symptoms in fruit of foliar-inoculated resistant lines, suggesting that foliar resistance may extend to fruit. However, in accordance with previous findings on developmental stage, seedling resistance and fruit disease incidence was found to vary within cultigens (Carvalho et al., 2013, Hopkins et al., 1993), suggesting different resistance mechanisms. Differences in fruit-level resistance have been suggested to be linked to fruit color: darker varieties corresponded to higher resistance (Hopkins et al., 1993), though this has not been confirmed in any further research; it is likely that dark rinds may mask symptoms, giving the appearance of resistance. However, in favor of greenhouse based screening, Hopkins and Thompson (2002) deemed extensive fruit screening as

useful but impractical. As far as the literature has demonstrated, a large-scale study of fruit resistance has not been conducted. There are, however, observations of fruit symptoms during the course of other inoculations (Bahar et al., 2009b, Walcott et al., 2003, Hopkins and Thompson, 2002b, Rane and Latin, 1992, Walcott et al., 2004, Burdman et al., 2005) and fruit disease incidence (Sowell and Schaad, 1979, Hopkins et al., 1993). In addition to these observations, there are studies that formally investigated fruit infection (Dutta et al., 2012, Carvalho et al., 2013, Frankle et al., 1993, Hopkins et al., 1993). In a commercial variety screen by Hopkins et al. (1993), fruit resistant lines were identified, but the resistance was considered insufficient to provide control under high disease pressure. A large-scale PI screen for watermelon fruit resistance has not been conducted.

Fruit infection occurs as *A. citrulli*, from infected leaf tissue, enter through stomata early in fruit development (Frankle et al., 1993). Based on the findings by Frankle et al. (1993), though, the fruit infection appears to be primarily governed by the accumulation of waxy cuticle. Over a five week period the percent decrease of diseased fruit correlated with the percent decrease of exposed stomata, with the fruit being most vulnerable (over 90% infection) during the first two weeks post anthesis (Frankle et al., 1993) This interestingly implies a barrier resistance rather than pathogen- or effector-triggered immunity. It can be ventured that genes that promote the early and rapid biosynthesis of cuticle will shorten the infection window, ultimately decreasing the percentage of infected fruit and diminishing seed infestation. Also, bactericides could be targeted during this shortened infection window (Frankle et al., 1993). Selecting for barrier resistance has the potential to be effective regardless of the strain, decreasing the danger of mutant or exotic strains overcoming resistance. From a breeding

standpoint, early selections for resistance may only require the selection of lines that develop waxy cuticles early in fruit development or, conversely, lines that have fewer fruit stomata.

Although fruit resistance would be valuable for producing marketable fruit and possibly disease-free seed notwithstanding foliar infection, the lack of research on fruit resistance in favor of foliar screening methods has three likely explanations: 1- large-scale screening at the fruit stage is resource-intensive (Hopkins and Thompson, 2002b); 2- foliar resistance, even if it doesn't correlate with fruit resistance, may sufficiently interrupt the disease cycle for disease control (Hopkins and Thompson, 2002b); 3- fruit inoculation methods have not been well established. Of course, foliar screening has provided a convenient way to test for resistance, but the research has not resulted in foliar resistant varieties. Despite fruit being the most crucial organ affected by BFB (Bahar et al., 2009b), fruit resistance remains vastly unexplored and the correlation to foliar resistance unknown; screening the USDA watermelon germplasm collection would potentially yield lines with fruit resistance.

4 CONCLUSION

Notwithstanding immense research effort to understand *A. citrulli*, numerous resistance screenings, and attempts at resistance breeding, there are no commercial watermelon varieties with resistance to BFB (Johnson et al., 2011). Because of seed circulation, BFB has become a worldwide problem for watermelon and other cucurbits (Latin and Hopkins, 1995, Schaad et al., 2003, Bahar and Burdman, 2010, Isakeit et al., 1997, Langston Jr et al., 1999, Tian et al., 2016, Carvalho et al., 2013). In the absence of resistant cultivars, alternative control measures such as seed treatments, field and greenhouse bactericide applications, and biocontrol all decrease *A. citrulli* and could be used in a BFB IPM program. However, the best control measure continues to be detection and exclusion of infested seed-lots using grow-out assays or PCR-based methods

(Hopkins and Levi, 2008, Bahar et al., 2008). Grow-out assays are inherently expensive and slow and PCR-based methods, while much faster and less expensive, can be have oversensitivity issues, although recent improvements have improved differentiation (Tian et al., 2016).

Thus far much of the early identified foliar resistance has been overcome by *A. citrulli* strain diversity (Walcott et al., 2000, Johnson et al., 2011, Bahar and Burdman, 2010) or the resistance hasn't held in new environments or on other plant developmental stages (Carvalho et al., 2013, Bahar et al., 2009b, Hopkins and Thompson, 2002b). Moreover, cultigens with identified BFB resistance are wild PIs with poor horticultural traits that will require extensive breeding to introgress resistance based on polygenic inheritance (Hopkins and Levi, 2008). In order to overcome these resistance screening pitfalls, screening studies should use a wide *A. citrulli* diversity, multiple group I and group II strains, and screen for resistance at the major developmental stages in production conditions.

Most of the watermelon screenings have focused on foliar resistance at the seedling- or flowering-stage. Fruit resistance would interrupt the disease cycle and allow for marketable fruit even when significant foliar infection exists and possibly protect the seed for infestation. Fruit resistance differs from foliar resistance in that the resistance is likely based on stomatal plugging by the cuticle (Frankle et al., 1993) and would thus be a barrier resistance and irrespective of the particular *A. citrulli* strain. Notwithstanding evidence of fruit resistance (Hopkins and Thompson, 2002b, Hopkins et al., 1993, Carvalho et al., 2013) and potential benefits, fruit resistance has been largely unexplored in the literature. Commercial varieties with fruit resistance would be highly valuable for the watermelon industry.

The primary objective of this is the identification of watermelon fruit resistance to BFB through an unprecedented large-scale screening the currently available watermelon PI collection

and a broad selection of commercial cultivars. Additionally, because fruit inoculation methods are not well defined in the literature, we tested variations of the dominant methods under field conditions in order to identify an optimal method. Finally, using the three-year screening data, we generated broad-sense heritability estimates for fruit resistance and simulated resource allocations for experiment optimization. The resistant cultigens identified in this study will be valuable for watermelon fruit resistance breeding and for research on fruit resistance mechanisms, genetic underpinnings, and inheritance.

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Chapter 2

Screening for Bacterial Fruit Blotch Resistance in Watermelon Fruit

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ABSTRACT

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* is a serious threat to the watermelon industry. Despite many foliar resistance screenings that have identified sources of resistance, there are currently no commercial watermelon lines with resistance. In this study, we conducted an immature fruit screening of 1,452 *Citrullus* spp. PI accessions and 19 cultivars under field conditions over three years. Due to high levels of missing data 841 cultigens had sufficient replication for analysis and among these lines, a subset of the possible resistant lines was tested at high replication over two years. We used a modified spray method to inoculate immature fruit in the field and rated 21-days post inoculation on a 0 to 9 scale based on the percentage of the surface that had disease symptoms and recorded the symptom type incidence. Bacterial fruit blotch symptoms progressed, with varying degrees, initially as raised bumps, to blotching, cracking, and internal necrosis. We found that the resistant lines rarely proceeded beyond raised bumps at the infection site and susceptible varieties had an overall higher incidence of the more advanced symptoms. Among the subset of 40 PIs identified during the first two years of screening and tested at high replication PI 494819 (*C. lanatus*), PI 596659 (*C. amarus*), PI 596670 (*C. amarus*), PI 490384 (*C. mucosospermus*), and PI 596656 (*C. amarus*) had disease rating of less than one, significantly more resistant than the commercial checks Charleston Gray; low variability within each PI; and relatively low advanced symptom incidence. These PIs are potential sources for resistance breeding programs and research focused on understanding fruit resistance mechanisms.

1 INTRODUCTION

Watermelon *Citrullus lanatus* var. *lanatus* [Thunb.] Matsum. & Nakai is a valuable crop grown on over 3.4 million hectares around the world in 2012 (Food and Agriculture Organization of the United Nations, 2014). In 2015, the US harvested 47,125 ha of watermelon at a total value of over $488 million dollars (USDA National Agricultural Statistics Service, 2017). Watermelon is part of the Cucurbitaceae family which includes many important crops such as melon (*Cucumis melo* L.), squash (*Cucurbita pepo* L.), and cucumber (*Cucumis sativus* L.).

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* (Schaad et al., 2008) is a major seed-borne disease that affects primarily watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) seedling and fruit production around the world (Latin and Hopkins, 1995, Schaad et al., 2003). The BFB causal agent was first isolated in 1965 at the Regional Plant Introduction Station, Experiment, GA from two watermelon plant introductions (PIs) that presented water-soaked lesions on their leaves (Webb and Goth, 1965). The first report of confirmed BFB in commercial watermelon fields was in the Mariana Islands in 1987 where it was coined “fruit blotch” (Wall and Santos, 1988). In 1989, it was found in watermelon fields in the United States (Hopkins, 1989); the initial outbreaks in South Carolina and Florida caused watermelon losses nearing 80% (Hopkins et al., 1993). BFB has since spread to most watermelon producing areas of the United States (Somodi et al., 1991, Wall et al., 1990, Hamm et al., 1997). Outbreaks of BFB can lead to a complete loss of production fields, and can cause serious damage, 5% to 50%, to nearby fields through secondary outbreaks (Latin and Hopkins, 1995). Because *A. citrulli* is a worldwide threat, contaminated plant import restrictions exist in the USA, China, and Europe (Tian et al., 2016).

Acidovorax citrulli can infect all growth stages of the watermelon plant: seeds, seedlings, foliage, flowers, and fruit (Latin and Hopkins, 1995). Seedling symptoms are water-soaked, brown lesions on the cotyledons and hypocotyl which often leads to plant death (Latin and Hopkins, 1995). Leaf lesions are often discreet, "small, dark brown and somewhat angular" (Latin and Hopkins, 1995) and tend to present along the major leaf veins (Hopkins et al., 1993). Infected plants are generally not defoliated, but rather leaf tissue becomes a reservoir for *A. citrulli* that later spread to developing fruit (Bahar et al., 2009b, Latin and Hopkins, 1995, Hopkins and Thompson, 2002, Frankle et al., 1993). Rane and Latin (1992) found that while the pathogen could be isolated from blotch margins on the fruit, it was not found in the peduncle and stems, suggesting that fruit infection does not appear to occur systemically through the vine. However, researchers studying group I strain mobility presented evidence suggesting that A. *citrulli* is able to infect and migrate in the xylem of seedlings (Bahar et al., 2009a). Watermelon fruit are most vulnerable to *A. citrulli* bacterium entering through stomata during the first five weeks post anthesis, prior to waxy cuticle stomatal plugging (Frankle et al., 1993). Fruit symptoms quickly progress from inconspicuous water-soaked lesions with irregular margins to expanded dark-green lesions that fissure, causing massive internal infection by secondary organisms, which leads to fruit collapse (Latin and Hopkins, 1995, Hopkins et al., 1993). Seeds are internally and externally infested with *A. citrulli* bacteria (Rane and Latin, 1992), completing the disease cycle. Infestation can occur in symptomatic fruit or asymptomatic fruit exposed to the pathogen (Hopkins et al., 1996, Carvalho et al., 2013) or through a blossom infestation pathway (Walcott et al., 2003) Seed contamination does not appear to be affected by storage under dry conditions over 12 months and would likely not be eliminated by longer-term storage (Hopkins et al., 1996). Indeed, we have observed infected seedlings from four-year-old seed

stored under cool, dry conditions. Insidious seed infestation continues to spread the pathogen around the world, representing a serious threat to the watermelon industry.

Currently, the most effective control measure is the exclusion of infected plants at seedling production facilities (Hopkins and Thompson, 2002, Hopkins et al., 1996). The USDA National Seed Health System gives guidelines and procedures for three detection methods: grow-out assay, seedling PCR, and ISHI Method (seed-based detection) (USDA National Seed Health System, 2017). The standard detection technique is a the seedling grow-out assay where 10,000 to 30,000 seeds per lot are grown for three weeks and compared to positive controls to determine infection (USDA National Seed Health System, 2017). Although effective, the grow-out method adds significant cost to seed production. As an alternative to, or in conjunction with, grow-out assays, numerous PCR detection methods have been developed (Walcott and Gitaitis, 2000, Walcott et al., 2006, Bahar et al., 2008, Ha et al., 2009, Tian et al., 2016) in order to rapidly and accurately test seed lots. Seed exclusion methods are a valuable part of a multipronged approach to limiting BFB outbreaks, and advancements in detection methods have made *A. citrulli* infested seed exclusion increasingly efficient.

Another control strategy is to limit the spread of the pathogen during transplant production through the use of copper-based bactericides and peroxyacetic acid (Hopkins, 1995, Hopkins et al., 2009), though some strains are showing copper insensitivity (Walcott et al., 2004). In the field, moderate chemical control can be achieved through the repeated application of copper containing products during early fruit development (Hopkins, 1991, Hopkins et al., 2009). Seed treatments to eliminate the pathogen have also been extensively explored with promising effectiveness (Hopkins et al., 1996, Sowell and Schaad, 1979, Wall, 1989, Hopkins et al., 2003, Feng et al., 2009a, Rane and Latin, 1992), but in some cases, there can be marked

decreased post-treatment germination (Feng et al., 2009a). However, despite detection efforts and control measures, sporadic BFB field outbreaks continue to cause significant damage to watermelon production (Hopkins et al., 2009).

Bacterial fruit blotch resistance would be a highly valuable in reducing current costly preventative measures and could be become an important part of integrated pest management (IPM), but despite the intensive efforts by many researchers, there are no resistant commercial watermelon lines available (Johnson et al., 2011). There have been many large-scale laboratory, greenhouse, and field screenings evaluating foliar resistance in watermelon (Hopkins et al., 1993, Hopkins and Thompson, 2002, Carvalho et al., 2013, Ma and Wehner, 2015, Hopkins and Levi, 2008, Sowell and Schaad, 1979) and melon (Bahar et al., 2009b, Wechter et al., 2011). Subsequent watermelon breeding efforts have shown that foliar resistance is polygenic (Hopkins and Levi, 2008). In contrast to foliar-focused studies, there have only been two minor watermelon fruit disease incidence screenings (Carvalho et al., 2013, Hopkins et al., 1993), fruit observations during a foliar resistance study (Hopkins and Thompson, 2002), several isolate pathogenicity studies involving fruit inoculations (Burdman et al., 2005, Sowell and Schaad, 1979), and a fruit resistance mechanism study (Frankle et al., 1993). Although fruit resistance would be valuable for producing marketable fruit and possibly disease-free seed in spite of foliar infection, the lack of research on fruit resistance in favor of foliar screening methods has three likely explanations: (1) large-scale screening at the fruit stage is resource-intensive (Hopkins and Thompson, 2002); (2) foliar resistance, even if it doesn't correlate with fruit resistance, may sufficiently interrupt the disease cycle for disease control (Hopkins and Thompson, 2002); (3) fruit inoculation methods have not been well established. Of course, foliar screening has provided a convenient way to test for resistance, but the research has not resulted in foliar

resistant varieties. Despite, fruit being the most crucial organ affected by BFB (Bahar et al., 2009b), fruit resistance remains vastly unexplored and the correlation to foliar resistance unknown; screening the USDA watermelon germplasm collection would potentially yield lines with fruit resistance

In this study, we sought to identify BFB fruit resistance through a broad screening of *Citrullus* spp. plant introductions and commercial cultivars under field conditions. A subset of lines demonstrating resistance during the initial screenings were tested under high replication in subsequent years. Lines demonstrating fruit resistance to BFB would be valuable breeding programs and future studies on fruit resistance mechanisms.

2 MATERIALS AND METHODS

2.1 *Acidovorax citrulli* Isolation and Identification

In 2015, we inoculated a combination of group I isolates, AU2, AAC200-30, and group II isolates, AAC94-21, AAC00-1, obtained from Dr. Ron Walcott at the University of Georgia (2014). In 2016 and 2017, we inoculated using a combination of four group I *A. citrulli* isolates (group identification described below) recovered from *Citrullus* spp. PI accessions planted in fields in Clinton, NC in early 2016. For isolation, symptomatic plants were field tested for *A. citrulli* using ImmunoStrip for *Acidovorax avenae* subsp. *citrulli* (Agdia, Inc., Elkhart, IN). The symptomatic leaves were surface-sterilized by immersion in a 1% sodium hypochlorite solution for three minutes and then washed twice with sterile distilled water (SDW) for 10 s. Small sections (~1 cm^2) of surface-sterilized leaf tissue were briefly macerated in a drop of (~10 µl) sterile dH_20 and set for 2 min to allow interior *A. citrulli* bacterium to flow out of the leaf tissue; 5ul of the bacterial solution was spread on nutrient agar (NA) (N9405 Nutrient Agar; Sigma-Aldrich, St. Louis, MO) plates and grown for 36 hr at 30°C in an incubator (Fisher 516D

Isotemp 500 Incubator; Fisher Scientific, Waltham, MA). Subsequently, twice, single cream-colored colonies (characteristic of *A. citrulli*) were transferred to NA plates and grown to create pure isolates. Isolates were confirmed as *A. citrulli* by again using ImmunoStrip for *Acidovorax avenae* subsp. *citrulli* (Agdia, Inc., Elkhart, IN) and stored as long-term stock solutions in 20% glycerol solutions in 1.5 microcentrifuge tubes at -80°C. Short-term isolate stocks were grown for 48 hr on NA and stored in a refrigerator at 4°C for immediate use and replaced monthly using the long-term stock solutions as isolate viability would gradually decrease over three months at 4°C.

In 2017, we determined the *A. citrulli* groups for the 2016 and 2017 field isolates using polymerase-chain reaction (PCR) primers reported by Zivanovic and Walcott (2017) based on group-specific type III secretion system sequence differences. For comparison, known isolates were used: group 1 – AAC200-30 and group 2- AAC00-1. For PCR, DNA was obtained by suspending *A. citrulli* cells from each isolate in 100µl of sterile distilled water and incubating at 95°C for 10mins. PCR reactions contained 5.3 µl dH2O, 3.5µl [10µM] forward primer (G2AcFwd, a group II-specific primer, or G12AcFwd, group I and II primer), 3.5 µl [10 µM] reverse primer, 1.5 µl DNA, and 5 µl GoTaq Green Master Mix (Promega, Madison, WI). PCR was conducted using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA) in accordance with temperatures described by Zivanovic and Walcott (2017): 95°C for 5 mins; 29 cycles of 95°C for 30 s, 55°C for 40 s, and 72°C for 30 s; 95°C for 30 s; 55°C for 40 s; and 72°C for 5 min. Electrophoresis (Run One Electrophoresis Cell; Embi Tec, San Diego, CA) on a 2% agarose gel precast with 1 µl GelRed Nucleic Acid Gel Stain, 10,000X (Biotium, Fremont, CA) was run at 100 V for 40 min. A 100bp DNA Ladder (New England Biolabs, Ipswitch, Massachusetts) was used to estimate amplicon band size: 291bp amplicon for group II-specific

primers (G2AcFwd/G12AcRev) and 254bp amplicon for group I and II-specific primers (G12AcFwd/G12AcRev) (Zivanovic and Walcott, 2017). Amplicons were submitted to the Genomic Sciences Laboratory at North Carolina State University for sequencing to confirm identity.

2.2 Inoculum Preparation, Inoculation Method, and Ratings

The morning of inoculations, we created inoculum by rinsing *A. citrulli* colonies grown for 48 hrs from NA plates using sterilized dH_20. Suspensions from each *A. citrulli* isolate were diluted to an OD600 of 0.25 [~10^8 colony forming units (CFU)/ml] using a spectrophotometer (UV160U; Shimadzu, Columbia, MD). Calibrated isolates were combined in equal portions to create the field inoculum and stored at shaded ambient field temperatures (~30°C) immediately prior to use.

Fruit inoculations started as soon as developing fruit were observed in the field and occurred over four inoculations dates at one-week intervals per testing block. It was critical to time the fruit development so as to inoculate during the window of highest vulnerability (>90% disease incidence), which was shown to be approximately 1 to 2 weeks post anthesis (Frankle et al., 1993). However, because of the number of plants to be inoculated, exact timing of fruit in the field was not feasible, so physiological immaturity markers were used to gauge fruit susceptibility: fruit size generally ranging 5-13 cm in diameter and a glossy, soft wax cuticle, typical of early maturing fruit (Figure 2.1). Immature fruit were identified, flagged using colored flags corresponding to the inoculation date, and the upper surface was sprayed with inoculum until run off. To decrease the possibility of escapes, inoculated fruits were resprayed 3 days later. Control fruit were selected randomly in the field from plants that presented two fruit at a similar growth stage and sprayed with dH_2O rinsed across sterile NA plates until runoff. Disease

severity was rated 21 days post inoculation using a 0 to 9 scale based on the percentage of the upper fruit surface showing symptoms. Multiple fruit from the same plant on the same inoculation date were averaged. We also recorded the symptom incidence: blotching, surface cracking, and internal necrosis.

2.3 Experimental Design and Field Practices

In the planting seasons of 2015, 2016, and 2017, we screened 1433 *Citrullus* spp. plant introductions (PIs) and 19 commercial cultivars, representing the 2014 available germplasm from the Plant Genetic Resources Unit in Griffin, Georgia and several commercial varieties for resistance to *A. citrulli* at the Horticultural Crops Research Station in Clinton, NC. In total, using the late Nov 2017 Germplasm Resource Information Network (GRIN) web server (https://www.ars-grin.gov/) nomenclature, there were 106 *Citrullus amarus,* 21 *Citrullus colocynthis,* 54 *Citrullus mucosospermus,* and 1271 *Citrullus lanatus*. We conducted the screenings using complete randomized block designs consisting of three screening blocks per year. In each screening block, each accession was replicated once and some commercial varieties were replicated 2 to 3 times.

In order to improve statistical confidence, in 2016 and 2017, a "retest" population composed of a subset of resistant lines from prior years that also had degrees of horticulturally acceptability and a "missing" population that had had excessive missing plots in the prior screening blocks were planted at higher replications in concurrent blocks. The retest and missing experiments consisted of 40 accessions and 4 cultivars replicated 12 times and 132 accessions replicated four times, respectively. Additionally, to detect segregation within cultigens, in 2017, we also tested an "elite" population consisting of 2 to 3 selfed progeny of the 15 most resistant accession from the 2016 retest and 4 cultigens. Each elite progeny line and cultigen was

replicated seven times. The retest, missing, and elite experiments were completely randomized designs.

In the spring, we direct seeded cultigens in single plant hills on raised beds covered with black plastic. The screening blocks were planted at two-week intervals, and missing plots were replanted two weeks after the initial planting. Each screening block consisted of thirty-two 61 m rows on 3.1 m centers with 1.2 m spacing between plants. Retest and missing experiments were planted on sixteen rows each and the elite experiment was planted on eight rows; row spacing was 3.1 m centers with 1.8 m spacing between plants. Plants were spiral trained once just prior to anthesis. The soil at the Horticultural Crops Research Station at Clinton is slightly acidic and sandy with little organic matter and receives ~121 cm annual rainfall with the majority occurring June through August (NCDA&CS, 2017). Fertilizer and water were provided via drip irrigation tubes under the plastic. Prior to planting, the fields were prepared with 10-8.3-4.4 (N-P-K) fertilizer at a rate of 561 kg ha^{-1}. After planting, fertilizer was regularly applied at 224 kg ha^{-1} of 13.5-0-19.8 (N-P-K) and 112 kg ha^{-1} of calcium and 15.5-0-0 9 (N-P-K). Pesticides were used as needed for insect control in accordance with best practices.

2.4 Statistical Analysis

Statistical analysis and data visualization were performed using the R environment (R Development Core Team, 2017). Mixed model analysis of disease severity was performed using ASREML-R 3.0 (Butler et al., 2009) on lines with at least two observations. The final model considered environmental effects as random and cultigen effects as fixed and was determined by adding random effects and comparing incremental models using the REML likelihood ratio tests provided by the 'asremlPlus' 2.0-12 (Brien, 2016) function *reml.lrt.asreml()*. We determined the best model to designate "Cultigen" as the sole fixed effect and the random effects to include

“Cultigen” by “Year”, “Block” nested in “Year,” “Cultigen” by “Block” nested in “Year,” and “Rating Date” nested in “Block” nested in “Year.” The mixed model is given here in ASREML-R notation: Average.rating ~ 1 + Cultigen, random=~ Cultigen:Year + Year/Block + Year/Block*Cultigen + Year/Block/Rating Date. However, to eliminate the nonsignificant random effect “Year,” the following ASREML-R notation was used: Average.rating ~ 1 + Cultigen, random=~ Cultigen:Year + Year:Block + Year:Block:Cultigen + Year:Block:Rating.Date. The predicted disease ratings for each cultigen and min, max, and average standard errors of the differences (SEDs) were calculated using the ASREML-R *predict()* function. In order to assess the significance of the fixed effect, Cultigen, a conditional Wald F-test was conducted using the ASREML-R *wald.asreml()* function with “sstype ='conditional'” and “dendf = 'numeric.'”

A Post-hoc Tukey HSD test (p = 0.05) was conducted on average disease severity on as subset of the most resistant and most susceptible lines identified in the advanced tests using the 'lsmeans' 2.26-3 package (LENTH, 2016) functions *lsmeans()* and *cld()* with “adjust = 'tukey'” on a mixed model analysis generated using 'lme4' 1.1-13 (Bates et al., 2015) *lme()* function.

3 RESULTS

3.1 *Citrullus* spp. Fruit Resistance to *A. citrulli*

Of the 1,452 *Citrullus* spp. tested over all tests in the three-year period, 1,357 cultigens had at least 2 observations and 841 had at least 4 or more observations and (Figure 2.4). Because of the challenges associated with testing immature fruit under field conditions and germination issues, there were significant amounts of missing data (Table 2.1; Figure 2.4; Appendix B). Among the 1,357 lines with at least 2 or more observations, the fixed effect “PI” was significant (Wald F-Test, p < 0.0001) and the predicted means for disease severity rating ranged from 0.08

to 9.32 and the SEDs were 1.63 average, 0.88 minimum, 2.23 maximum (Appendix B). Most lines appeared to have moderate, however unacceptable, resistance with an average disease rating of 3.3 and a standard deviation of 1.4. Overall, 273 cultigens had disease ratings of less than 2, suggesting potential resistance; however, many of these lines had low replications and would require additional testing to validate the results presented.

In the post-hoc pairwise comparison of a subset of lines in the advanced testing, the average ratings ranged from 0.5 to 5.1 and the three-year study replications ranged from 13 to 35 with a median of 17 (Table 2.4). The most resistant lines (average rating < 2) were composed of *Citrullus lanatus, Citrullus amarus,* and *Citrullus mucosospermus* and were collected widely across Africa. The commercial checks Sugar Baby and Mickylee rated at a 2.2 and 2.6 and were statistically indistinguishable from the most resistant cultigen, PI 494819, but Starbrite F1 and Charleston Gray, rated at 4.1 and 4.3, were significantly different (Tukey, p = 0.05). In contrast to the commercial checks, resistant lines with an average rating less than 1 had relatively narrow rating ranges. For example, all 22 disease ratings for PI 494819, the most resistant line, were consistently 0 or 1, suggesting a strong and consistent resistance. Across all cultigens, as the average rating increased, the rating range also increased such that the minimum ratings ranged 0 to 1.5 and the upper ratings ranged 6 to 9. However, the portion of higher ratings increased in more susceptible lines, e.g., Charleston Gray and PI 482344 which both ranged 0 to 8 but had average ratings of 4.3 and 1.7, respectively. The most susceptible lines included in the post hoc test, PI 635668 and PI 629108, are the cultivars known as ‘Arikara’ and ‘Golden Honey,’ respectively.

The infection generally proceeded as bumps, likely representing *A.* citrulli initial entry points, to blotching and cracking, and finally internal necrosis and fruit collapse (Figure 2.2).

All lines generally had some level of raised bumps associated with the inoculated area which were often innocuous, especially when not associated with blotching and discoloration, and could only be confirmed by touching the infected area. Unsurprisingly, the most susceptible lines rapidly progressed to internal necrosis and had increased symptom incidence combinations. In contrast, resistant lines infrequently advanced beyond the bump infection stage, and when internal necrosis occurred, it tended to be restricted to the rind.

3.2 *Acidovorax citrulli* isolate identification

Four *A. citrulli* isolates in 2016 and two isolates 2017 were recovered from nonadjacent infected PIs randomly grown at the Horticultural Crops Research Station in Clinton, NC were all found to be group I isolates. Isolates from the infected tissue tested positive for using ImmunoStrips (Agdia, Inc., Elkhart, IN) (data not shown). Subsequently, marker analysis on pure isolates using primers described by Zivanovic and Walcott (2017) yielded bands specific to both group I and group II isolates and no band for the primers specific only to group II isolates; the controls: group II isolate, AAC00-1, and group I isolate, 200-30, each produced the expected bands (figure 2.5). Further confirming the isolate grouping, isolate and control group-specific amplicon sequences matched the expected *A. citrulli* sequences at >99% sequence homology (Figure 2.6, Figure 2.7).

4 DISCUSSION

4.1 Fruit Resistance to *A. citrulli*

We conducted a three-year field screening of *Citrullus* spp. cultigens for fruit resistance to *A. citrulli* and identified five lines with high resistance: PI 494819, PI 596659, PI 596670, PI 490384, and PI 596656. These resistant lines had relatively lower average ratings and variation. The lack of statistical separation between PI 494819, the most resistant line, and Sugar Baby, our

most resistant cultivar, using TUKEY pairwise comparisons (p = 0.5) illustrates the high variation inherent in this field testing method. However, when the post hoc analysis is conducted using the less conservative Fischer protected test of LSD, there is mean separation between PI 494819 and Sugar Baby (data not shown). In addition, it should be considered that over the course of the three-year study PI 494819 was rated 22 times for disease severity as a 0 or 1 and only one fruit had noticeable blotching and internal necrosis. Overall, our top resistant lines didn't have any symptoms, symptoms didn't advance beyond raised bumps, or when symptoms such as blotching and cracking did occur, it was minimal. However, none of the lines from the advanced tests were completely immune, but even minor symptoms, especially bumps, could be inconspicuous enough to be commercially acceptable. Interestingly, Hopkins et al. (1993) suggested that fruit resistance may be associated with dark rind color. While we did not specifically record fruit color during the course of this study, slight bumps and blotching were more difficult to see on darker skinned fruit. It is doubtful that rind color correlates to resistance per se, rather resistant lines with dark-colored rinds would likely appear more resistant than the exact same lines with light-colored rinds. Unfortunately, as has been seen in other studies (Ma and Wehner, 2015, Hopkins et al., 1993, Hopkins and Thompson, 2002), we found that the commercial cultivars lack sufficient resistance and that our top resistant lines tend to be wild accessions with undesirable horticultural traits. If fruit resistance inheritance is complex, as has been shown for seedling resistance (Hopkins and Levi, 2008), overcoming linkage drag through a backcross breeding scheme would require extensive progeny testing to obtain resistant lines with acceptable horticultural traits. Regardless, the top five resistant PI's represent sources of fruit resistance that could be introgressed into commercially acceptable lines.

During the course of the three-year study, we screened 1452 cultigens, but only 847 had at least four observations and only a small subset of lines with resistance from the 2015 and 2016 screening blocks were chosen to be included in the advanced testing in 2016 and 2017 (Table 2.5). Because the 2017 screening blocks were conducted concurrently to the last advanced lines test and the data was not considered for selecting resistant lines for advanced testing. Thus, the lines and commercial checks presented in table 2.4 are not comprehensive but rather provide a starting point for future fruit resistance breeding and confirm that fruit resistance exists within the *Citrullus* spp. germplasm. Other cultigens with low disease ratings presented in Appendix B are a potential pool for resistance but would require further testing.

4.2 *Acidovorax citrulli* Among the *Citrullus* spp. PI Accessions

In the summer of 2017, three-week-old seedlings ranging from cotyledon to three-leaf stage were visually evaluated for BFB infection. Of the 1500 single-plant hills, 1357 had emerged three weeks after planting and 575 (>%40) had visual symptoms of blotch (Figure 2.3). Although, our observations of infected lines within the PI collection are limited to single plants and not replicated, we did notice similar widespread infection in all other screening fields and a germplasm planting in the greenhouse (unpublished data). Similarly, Sowell and Schaad (1979) reported symptoms on 110 of 740 watermelon PIs, and the first known *A. citrulli* isolates came from *Citrullus lanatus* PIs (Webb and Goth, 1965, Schaad et al., 1978). In our case, it is conceivable that BFB could have spread from a few infected plants throughout the field, but this scenario is unlikely in that the 4 ft spacing between plants on plastic would limited cross infection. Although there was a soy rotation in 2016 and multiple herbicide applications and tilling, the field had been planted for inoculations assays in 2015. It is possible that infested watermelons surviving from 2015 seed may have contributed to increased disease incidence in

2017. However, we were unable find any literature indicating that *A. citrulli* can overwinter in soil. Even allowing for improbable spread from volunteers and other infected accessions and *A. citrulli* overwintering in the soil, our observations presented here suggest the rather dire view that a large minority of the *Citrullus* spp. PI collection is infested with *A. citrulli*. This infestation continues to pose a serious threat to researchers and breeders interested in accessing the *Citrullus spp.* diversity.

In our study, we used a combination of group I and group II isolates in 2015 and, in 2016 and 2017, four group I isolates from watermelon PI seedlings in Clinton, NC. Given the reported predominance of group II isolates on watermelons (Walcott et al., 2004) and the general association of group I strains with non-watermelon cucurbits (Eckshtain-Levi et al., 2016), we were surprised to find that the four isolates recovered in 2016 and two isolates not used for inoculum isolated in 2017 were all part of group I; we had expected a bias for group II isolates. Our observations were similar to other studies that identified regional grouping: in China where all 14 isolates from watermelon typed as group I (Feng et al., 2009b), and in Brazil where 66/67 isolates were group I, regardless of host (Silva et al., 2016). Admittedly, our sample size is too small to make any conclusion about *A. citrulli* group population dynamics in the *Citrullus* spp. PI collection. Interestingly, as far as investigated, there isn't published data on describing *A. citrulli* in the *Citrullus* spp. PI collection despite several papers that characterized strains collected around the world (Silva et al., 2016, Walcott et al., 2004, Eckshtain-Levi et al., 2014, Zivanovic and Walcott, 2017, Burdman et al., 2005, Feng et al., 2009b, O'Brien and Martin, 1999, Eckshtain-Levi et al., 2016). Our four group I isolates were collected from random and nonadjacent locations, but we did not characterize them beyond grouping. It is possible that our isolates could be the same or closely related, which would depend on possible predominance of

certain isolates in the *Citrullus* spp. PI collection. Characterizing *A. citrulli* isolates in the *Citrullus* spp. PI collection would facilitate a greater understanding of *A. citrulli* diversity and provide additional strains for broadening screening studies.

Fruit resistance to BFB would be very valuable for growers by allowing them to produce marketable fruit in the presence of a BFB outbreak. However, crucial for seed producers, the question as to whether or not infecting resistant fruit results in infested seed remains to be studied. The two primary seed infestation pathways are direct fruit infection (Latin and Hopkins, 1995, Hopkins and Thompson, 2002), as conducted in this study, and blossom infection (Walcott et al., 2003). Representing an insidious alternative infection pathway, Walcott et al. (2003) demonstrated that blossom infection can result in asymptomatic fruit with infested seed and pulp (Walcott 2003). It is unlikely that a fruit resistance mechanism that may be cuticle-based would preclude the blossom infestation pathway. As for direct inoculum exposure, (Hopkins and Thompson 2002), testing seed transmission in various cucurbits found that all cucurbit fruit misted with inoculum resulted in seed infestation. Moreover, symptomatic and adjacent asymptomatic fruit both had infested seeds, although the incidence was very minimal in the latter (Hopkins et al., 1996). However, other studies found that fruit of some lines had lower fruit disease incidence (Hopkins et al., 1993, Carvalho et al., 2013), but no effort was made to determine seed infestation rates. Similarly, in our study, our resistant lines had minimal or no observable symptoms, but we did not evaluate the degree of seed infestation. Although fruit resistance that prevents seed infestation would be valuable to seed producers, the correlation between our observed resistance and the degree of seed infestation remains uninvestigated.

4.3 Improving Fruit Resistance Screening

Our inoculation method, a variation of the method used by Hopkins and Thompson (2002a), was highly variable but produced consistent results over the three-year study. We found that rating 21 dpi allowed the infection to become severe in susceptible cultigens and generally matched fruit maturity, which mimicked natural BFB disease progression in a grower's field. Our decision to use a higher inoculum concentration than other screening studies, 10^8 CFU/ml, and inoculate twice with an isolate mixture was based on the rational used by Wechter et al. (2011): decrease the likelihood of escapes and ensure that identified resistance would be strong enough to cope severe disease conditions in the field. Our disease severity ratings for Sugar Baby and Charleston Gray, though not statistically separated in our TUKEY test ($p = 0.5$) (Table 2.4), do correspond to resistant and susceptible rankings for these cultivars in in other studies (Hopkins et al., 1993, Carvalho et al., 2013).

In our study, we had excessive missing data for a number of lines (Fig. 2.4; Table 2.1) which was due to a number factors: difficulty finding immature fruit in the field, non-germination among some PIs, loss of plants to disease, and extreme earliness or lateness in fruiting. Future resistance breeding could mitigate some of the missing fruit issues by high-replication testing of a narrow set of lines with similar fruit-timing. The inoculation protocol presented in this study did produce consistent results in a natural disease setting and, although labor-intensive, could be practical for large-scale breeding operations.

Each BFB screening study should be considered based on the conditions, methodology, and isolates used. Indeed, discrepancies in resistance results using lines based on prior screening studies are partially explained by differences in isolate diversity and testing conditions (Walcott et al., 2000). Further complicating resistance screening, there is variable aggressiveness within

the isolate groups (Eckshtain-Levi et al., 2014), a strong environmental effect (Hopkins and Thompson, 2002) and an effect of plant age and organ being tested (Carvalho et al., 2013, Bahar et al., 2009b). Ideally, a test would be conducted under natural growing conditions over multiple environments, as done in this study, and with a wide array of isolates so as to not select for resistance to particular isolate. Our lack of wide diversity, specifically only group I isolates in 2016 and 2017, is certainly a limitation of this research; however, because fruit resistance may rely on a cuticle-based barrier resistance (Frankle et al., 1993) rather than an effector-dependent resistance reaction (Eckshtain-Levi et al., 2014), the resistance may be irrespective of the inoculum diversity. Frankle et al. (1993) found that after five weeks, most stomata, the main ingress route for the pathogen, were plugged by cuticle and that this correlated to nearly zero disease incidence in Charleston Gray. In our study, that highly resistant lines either showed no or very little disease progression could be because of stomatal plugging completely prevents or severely limits inoculum into fruit. This assertion requires more testing but is supported by observations that mature fruit injection assays, which bypassed cuticle barriers, caused severe disease symptoms in Sugar baby and Charleston Gray (Chapter 3). Moreover, the wax (soft vs hard; glossy vs dull) was the best indicator of fruit vulnerability to infection during our research (Figure 2.1), suggesting that resistance is associated with some change in cuticle during development. Correlation between cuticle formation and disease resistance in our top resistant lines compared to our susceptible lines has not been investigated, but, if a relationship does exist, fruit screening for early stomatal plugging could indirectly select for fruit resistance to BFB. In addition, fruit resistance would enhance other control measures. For example, Frankle et al. (1993) proposed that controlling the BFB could be accomplished by applying bactericides until stomatal plugging occurs. Cultigens that have early stomatal plugging could also have shorter

infection vulnerability windows, thus making bactericides more effective. Such a cuticle study could be conducted rapidly under controlled greenhouse conditions by measuring stomatal plugging days post pollination and without having to handle *A. citrulli*. Lines that were found to rapidly accumulate cuticle could be later tested under using the field protocol in this study.

5 CONCLUSION

In this study, we established that strong fruit resistance exists in the watermelon germplasm, a particularly important trait for growers, but we did not examine seed transmission in these resistant lines. That could be accomplished in follow-on research simply using established seed infestation detection methods (USDA National Seed Health System, 2017) on fruit resistant lines. Fruit resistance presented here is useful for growers interested in producing marketable fruit; however, resistant lines that also minimized or prevented seed infestation would be extremely useful for seed producers and warrants further research.

The field screening method used in this study had high variation that could be lessened using more uniform lines. While the environment was a large source of the variation (Chapter 4), ultimately, the PI collection fruit diversity made this study particularly challenging by forcing us to use multiple inoculation dates in the same block to capture fruit from late- and early-fruiting lines, and fruit diversity made our fruit age estimates based on physiological markers inexact. This variation would be somewhat alleviated by using lines with similar fruit-timing and fruit development, as would be expected in a breeding population.

As an alternative to our screening protocol, because fruit resistance may be based on stomatal plugging (Frankle et al., 1993), future studies using our resistant and susceptible lines could demonstrate a correlation between fruit resistance and stomatal plugging or fewer stomata or both. It can be ventured that genes that promote early stomatal plugging or produce fewer

stomata will shorten the infection window and reduce the amount of inoculum entering the fruit, ultimately decreasing the percentage of infected fruit and possibly diminishing seed infestation. Selecting for barrier resistance has the potential to be effective regardless of the strain, decreasing the danger of mutant or exotic strains overcoming resistance. From a breeding standpoint, early selections for resistance may only require the selection of lines, possibly under controlled greenhouse conditions with exact pollination dates, that develop waxy cuticles early in fruit development or lines that have fewer fruit stomata. Cuticle-based fruit resistance would bypass many of the environmental challenges we had in the field and eliminate the need to handle *A. citrulli* until the results require validation using the field methods presented here.

We identified PIs with fruit resistance to BFB among the *Citrullus* spp. PI collection and commercial checks in the largest fruit resistance screening to date. However, the five most resistant lines represent a small subset of the possible resistant lines we observed in 2015 and 2016. With the additional 2017 screening data, there are many other candidate resistant PIs with moderate replication that could also be sources of resistance but would need to be confirmed in high-replication testing.

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Table 2.1 Description and number of observations for each BFB resistance screening experiments by year

Line	Year	Observed plants	Projected plants	Replications	No. lines[w]
Rep1	2015	322	1500	1[x]	1452
Rep2	2015	517	1500	1	1452
Rep3	2015	635	1500	1	1452
Missing 2016	2016	224	528	4	132
Retest 2016	2016	279	528	12	44
Rep4	2016	784	1500	1	1452
Rep5	2016	666	1500	1	1452
Rep6	2016	806	1500	1	1452
Retest 2017	2017	196	528	12	44
Elite Self's 2017	2017	115	259	7	19[y]
Missing 2017	2017	9[z]	528	4	132
Rep7	2017	656	1500	1	1452
Rep8	2017	657	1500	1	1452
Rep9	2017	378	1500	1	1452
Total		6244	16015		

[w]Plant introductions and commercial varieties
[x]Commercial lines in Rep1-9 were planted at higher replications
[y]Four commercial lines and selfed progeny from 15 PIs
[z]Missing 2017 had very low germination rates

Table 2.2. Cultigens replications in the 2016 & 2017 high-replication tests

Line	Species	Replications		
		Retest 2016	Retest 2017	Elite 2017
PI 500312	Citrullus lanatus	10	10	9
PI 183123	Citrullus lanatus	10	-	-
PI 482365	Citrullus lanatus	8	9	13
PI 169268	Citrullus lanatus	6	-	-
PI 176499	Citrullus lanatus	10	-	-
PI 182178	Citrullus lanatus	7	-	-
PI 169258	Citrullus lanatus	4	-	-
PI 278044	Citrullus lanatus	3	-	-
PI 635657	Citrullus lanatus	3	-	-
PI 512356	Citrullus lanatus	10	-	-
PI 500319	Citrullus lanatus	7	3	4
PI 176909	Citrullus lanatus	6	-	-
PI 505587	Citrullus lanatus	11	7	16
PI 254622	Citrullus lanatus	7	-	4
PI 500349	Citrullus lanatus	8	6	14
PI 532723	Citrullus mucosospermus	11	4	3
PI 596662	Citrullus amarus	6	-	-
PI 277988	Citrullus lanatus	7	-	-
PI 549160	Citrullus lanatus	8	3	-
PI 293766	Citrullus lanatus	7	-	-
PI 490376	Citrullus lanatus	6	-	-
PI 177326	Citrullus lanatus	7	-	-
PI 635668	Citrullus lanatus	7	-	-
PI 172790	Citrullus lanatus	6	-	-
PI 629108	Citrullus lanatus	6	5	-
PI 385964	Citrullus lanatus	3	6	-
PI 357739	Citrullus lanatus	4	-	-
PI 658554	Citrullus lanatus	5	1	3
PI 307750	Citrullus lanatus	8	-	-
PI 660975	Citrullus lanatus	6	-	-
Desert King	Citrullus lanatus	4	-	-
PI 381717	Citrullus lanatus	7	-	-
PI 250146	Citrullus lanatus	4	-	-
PI 164709	Citrullus lanatus	6	-	-
PI 325248	Citrullus lanatus	7	1	4
PI 270143	Citrullus lanatus	4	-	-
C. Gray	Citrullus lanatus	5	4	2

Table 2.2. Continued.

Line	Species	Replications		
		Retest 2016	Retest 2017	Elite 2017
Starbrite	Citrullus lanatus	4	-	2
PI 612462	Citrullus lanatus	7	-	-
PI 534533	Citrullus lanatus	1	1	7
PI 482314	Citrullus lanatus	-	7	-
PI 596656	Citrullus amarus	-	8	-
Sugar Baby	Citrullus lanatus	-	5	-
PI 169288	Citrullus lanatus	-	1	-
PI 381703	Citrullus lanatus	-	5	5
PI 560020	Citrullus mucosospermus	-	10	-
PI 534583	Citrullus lanatus	-	3	-
PI 381701	Citrullus lanatus	-	2	-
PI 482347	Citrullus lanatus	-	10	13
PI 596670	Citrullus amarus	-	10	1
PI 490384	Citrullus mucosospermus	-	8	-
PI 482344	Citrullus lanatus	-	8	3
PI 490380	Citrullus mucosospermus	-	5	-
PI 560015	Citrullus mucosospermus	-	10	-
PI 490379	Citrullus mucosospermus	-	3	-
PI 482276	Citrullus amarus	-	1	-
PI 596668	Citrullus amarus	-	7	-
PI 494819	Citrullus lanatus	-	7	9
PI 596659	Citrullus amarus	-	5	-
PI 169249	Citrullus lanatus	-	2	-
PI 494816	Citrullus lanatus	-	7	-
PI 271774	Citrullus lanatus	-	1	-
PI 596666	Citrullus amarus	-	2	-
PI 482296	Citrullus lanatus	-	3	-
PI 482273	Citrullus amarus	-	1	-
C. Sweet	Citrullus lanatus	-	-	2
Regency	Citrullus lanatus	-	-	1
PI 357656	Citrullus lanatus	6	-	-
Mickylee	Citrullus lanatus	9	5	-
PI 537467	Citrullus lanatus	3	-	-
PI 512401	Citrullus lanatus	5	-	-

Table 2.3. Cultigen replications in the 2016 and 2017 missing tests

		Replications	
Line	Species	Missing 2016	Missing 2017
PI 370424	Citrullus lanatus	2	-
PI 266028	Citrullus lanatus	3	-
PI 658680	Citrullus lanatus	4	-
PI 593357	Citrullus lanatus	3	-
PI 179885	Citrullus lanatus	2	-
PI 220779	Citrullus lanatus	3	-
PI 536464	Citrullus lanatus	1	-
PI 386014	Citrullus colocynthis	3	-
PI 278057	Citrullus lanatus	2	-
PI 482264	Citrullus lanatus	1	-
PI 379243	Citrullus amarus	3	-
PI 512369	Citrullus lanatus	4	-
PI 482269	Citrullus lanatus	1	-
PI 164708	Citrullus lanatus	2	-
PI 169295	Citrullus lanatus	1	-
PI 277279	Citrullus lanatus	3	-
PI 186489	Citrullus mucosospermus	4	-
PI 169254	Citrullus lanatus	3	-
PI 482330	Citrullus lanatus	2	-
PI 518612	Citrullus lanatus	3	-
PI 482348	Citrullus lanatus	4	-
PI 169288	Citrullus lanatus	2	-
PI 512377	Citrullus lanatus	2	-
PI 512351	Citrullus lanatus	3	-
PI 254736	Citrullus mucosospermus	2	-
PI 593364	Citrullus lanatus	4	-
PI 593386	Citrullus lanatus	1	-
PI 176491	Citrullus lanatus	4	-
PI 178873	Citrullus lanatus	4	-
PI 612470	Citrullus lanatus	1	-
PI 173888	Citrullus lanatus	1	-
PI 271747	Citrullus lanatus	2	-
PI 635700	Citrullus lanatus	1	-
PI 169275	Citrullus lanatus	1	-
PI 293765	Citrullus lanatus	1	-
PI 357737	Citrullus lanatus	1	-
PI 482335	Citrullus amarus	3	-

Table 2.3. Continued.

Line	Species	Replications	
		Missing 2016	Missing 2017
PI 560007	Citrullus mucosospermus	2	-
PI 549161	Citrullus colocynthis	1	-
PI 482351	Citrullus lanatus	2	-
PI 277999	Citrullus lanatus	3	-
PI 306366	Citrullus lanatus	2	-
PI 379248	Citrullus lanatus	1	-
PI 344395	Citrullus lanatus	1	-
PI 357704	Citrullus lanatus	1	-
PI 169265	Citrullus lanatus	1	-
PI 275631	Citrullus lanatus	2	-
PI 276445	Citrullus lanatus	3	-
PI 193964	Citrullus lanatus	1	-
PI 171585	Citrullus lanatus	2	-
PI 512331	Citrullus lanatus	3	-
PI 500303	Citrullus amarus	2	-
PI 278049	Citrullus lanatus	3	-
PI 277981	Citrullus lanatus	2	-
PI 635647	Citrullus lanatus	2	1
PI 431579	Citrullus lanatus	1	-
PI 593366	Citrullus lanatus	1	-
PI 182180	Citrullus lanatus	3	-
PI 482319	Citrullus amarus	2	-
PI 253174	Citrullus lanatus	2	-
PI 299379	Citrullus amarus	1	-
PI 344066	Citrullus lanatus	1	-
PI 222137	Citrullus lanatus	2	-
PI 536458	Citrullus lanatus	1	-
PI 222711	Citrullus lanatus	4	-
PI 177327	Citrullus lanatus	1	-
PI 173670	Citrullus lanatus	1	-
PI 271779	Citrullus amarus	1	-
PI 381736	Citrullus lanatus	4	-
PI 212094	Citrullus lanatus	1	-
PI 185030	Citrullus lanatus	1	-
PI 482298	Citrullus amarus	1	-
PI 381737	Citrullus lanatus	1	-
PI 295843	Citrullus amarus	2	-

Table 2.3. Continued.

Line	Species	Replications	
		Missing 2016	Missing 2017
PI 525088	Citrullus lanatus	1	-
PI 271983	Citrullus lanatus	2	-
PI 357728	Citrullus lanatus	1	-
PI 482355	Citrullus amarus	1	-
PI 601101	Citrullus lanatus	3	-
PI 184800	Citrullus mucosospermus	3	-
PI 629102	Citrullus lanatus	1	-
PI 181743	Citrullus lanatus	1	-
PI 172788	Citrullus lanatus	1	-
PI 593368	Citrullus lanatus	1	-
PI 500302	Citrullus lanatus	2	-
PI 254624	Citrullus lanatus	1	-
PI 635631	Citrullus lanatus	3	-
PI 368516	Citrullus lanatus	1	-
PI 482316	Citrullus amarus	1	-
PI 635660	Citrullus lanatus	1	-
PI 381696	Citrullus lanatus	2	-
PI 182181	Citrullus lanatus	1	-
PI 536459	Citrullus lanatus	2	-
PI 482352	Citrullus lanatus	3	-
PI 278019	Citrullus lanatus	2	-
PI 306782	Citrullus mucosospermus	3	-
PI 233556	Citrullus lanatus	1	-
PI 476326	Citrullus lanatus	1	-
PI 536452	Citrullus lanatus	2	-
PI 228238	Citrullus lanatus	1	-
PI 183299	Citrullus lanatus	2	-
PI 464872	Citrullus lanatus	2	-
PI 271981	Citrullus lanatus	1	-
PI 278021	Citrullus lanatus	1	-
PI 211915	Citrullus lanatus	1	-
PI 635726	Citrullus lanatus	2	-
PI 278062	Citrullus lanatus	2	-
PI 482336	Citrullus amarus	1	-
PI 357752	Citrullus lanatus	-	1
PI 254431	Citrullus lanatus	-	1
PI 420320	Citrullus lanatus	-	1

Table 2.3. Continued.

Line	Species	Replications	
		Missing 2016	Missing 2017
PI 482353	Citrullus lanatus	-	1
PI 512395	Citrullus lanatus	-	2
PI 525083	Citrullus amarus	-	1
PI 216029	Citrullus lanatus	-	1
PI 482337	Citrullus lanatus	4	-
PI 482347	Citrullus lanatus	3	-
PI 537465	Citrullus lanatus	3	-
PI 635683	Citrullus lanatus	2	-
PI 180278	Citrullus lanatus	3	-
PI 601228	Citrullus lanatus	1	-
PI 635662	Citrullus lanatus	1	-
PI 512348	Citrullus lanatus	1	-

Table 2.4. Disease ratings, statistical groupings and symptom incidence for a subset of cultigens selected for high resistance and susceptibility during the three-year screening and tested at high replication in the 2016 & 2017 retests and elite test.

									Symptom Incidence[u]		
Cultigen	Taxonomy[z]	Origin[z]	Disease Rating[y]	S.E.[y]	Group[x]	No.[w]	Range	No. ≤ 2[v]	Blotching	Cracking	Necrosis
PI 494819	lanatus	Zambia	0.5	0.5	a	22	0-1	22	1	0	1
PI 596659	amarus	South Africa	0.7	0.5	ab	14	0-2.8	13	4	1	1
PI 596670	amarus	South Africa,	0.8	0.5	ab	18	0-2	18	1	1	1
PI 490384	mucosospermus	Mali	0.8	0.6	ab	14	0-1.5	14	0	1	0
PI 596656	amarus	South Africa	0.9	0.6	ab	14	0-3.5	13	5	0	1
PI 482347	lanatus	Zimbabwe	1.1	0.5	ab	31	0-3.8	29	0	2	0
PI 549160	lanatus	Chad	1.2	0.5	ab	17	0-6	16	2	2	1
PI 596668	amarus	South Africa, Transvaal	1.2	0.6	ab	14	0-6	10	1	0	1
PI 532723	mucosospermus	Zaire, Bas-Zaire	1.3	0.5	ab	24	0-4.2	22	1	0	0
PI 500312	lanatus	Zambia	1.5	0.5	ab	36	0-6	29	2	0	1
PI 500349	lanatus	Zambia	1.5	0.5	abc	32	0-7	28	0	0	3
PI 560015	mucosospermus	Nigeria, Oyo	1.5	0.6	abcd	15	0-6	13	1	1	0
PI 500319	lanatus	Zambia	1.7	0.6	abcd	17	0-9	13	2	1	2
PI 482344	lanatus	Zimbabwe	1.7	0.5	abcd	18	0-8	15	3	1	1
PI 482365	lanatus	Zimbabwe	1.8	0.5	abcd	35	0-6	30	2	5	0
Sugar Baby	lanatus	NA	2.2	0.6	abcde	13	0-6	10	0	1	2
Mickylee	lanatus	NA	2.6	0.5	abcdef	23	0-7	10	3	2	4
PI 176499	lanatus	Turkey, Eskisehir	3.6	0.6	bcdef	15	1-8.5	6	2	1	1
Starbrite F1	lanatus	NA	4.1	0.6	cdef	13	0-8	3	3	4	3
PI 385964	lanatus	Kenya	4.3	0.6	def	14	1-9	4	1	1	1
C. Gray	lanatus	NA	4.3	0.5	def	17	0-8	3	2	1	3
PI 635668	lanatus	U.S., Wyoming	4.8	0.6	ef	13	1.5-8	3	4	1	7
PI 629108	lanatus	U.S., California	5.1	0.5	f	17	0-8	4	3	1	5

[z]According to the late Nov 2017 Germplasm Resource Information Network (GRIN) web server (https://www.ars-grin.gov/).
[y]Disease ratings and standard errors (S.E.) using the ‘lsmeans’ 2.26-3 package (Lenth, 2016) on a mixed model generated using ‘lme4’ 1.1-13 (Bates et al., 2015).
[x]Disease ratings that do not share a letter are significantly different at $P \leq 0.05$.
[w]The total number of observations for each cultigen over 2015-2017.
[v]The number of observations with a rating ≤ 2.
[u]The number of fruit exhibiting any noticeable degree of symptoms other than blistering. Fruit generally exhibited a combination of symptoms. Blotching refers to fruit surface discoloration; cracking indicates that the outer surface was broken; and necrosis means that the infection had caused internal necrosis.

Figure 2.1. Fruit exhibiting cuticle and color stages used for determining maturity for fruit inoculations. The dark-colored mature fruit (< three weeks post anthesis) has a dull, hard wax cuticle that repelled spray inoculum and seldom exhibit disease symptoms. The light-colored, immature fruit (< two weeks post anthesis) has a glossy, soft wax cuticle that adhered spray inoculum and would develop symptoms.

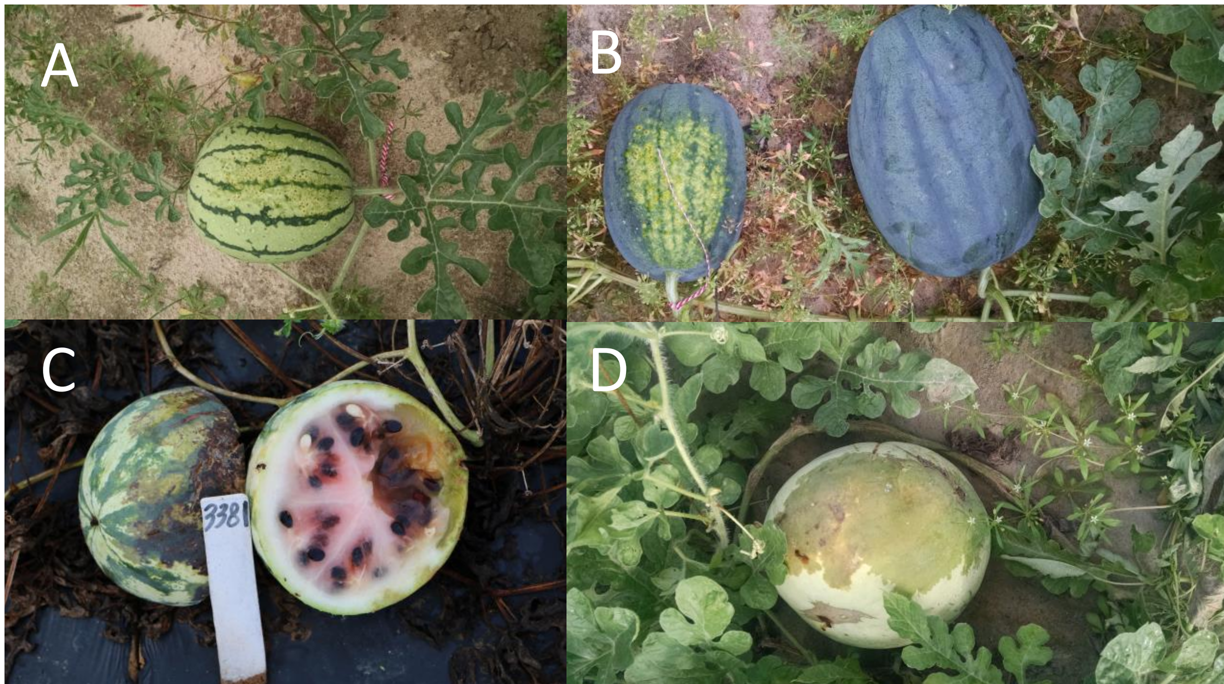

Figure 2.2. Bacterial fruit blotch symptoms observed during ratings: (A) bumps and minor blotching; (B) bumps with major blotching; (C) major bumps, blotching, cracking, and internal necrosis; (D) minor bumps and major blotching.

Figure 2.3. Bacterial fruit blotch symptoms observed on three-week old PI seedlings during the 2017 resistance screening.

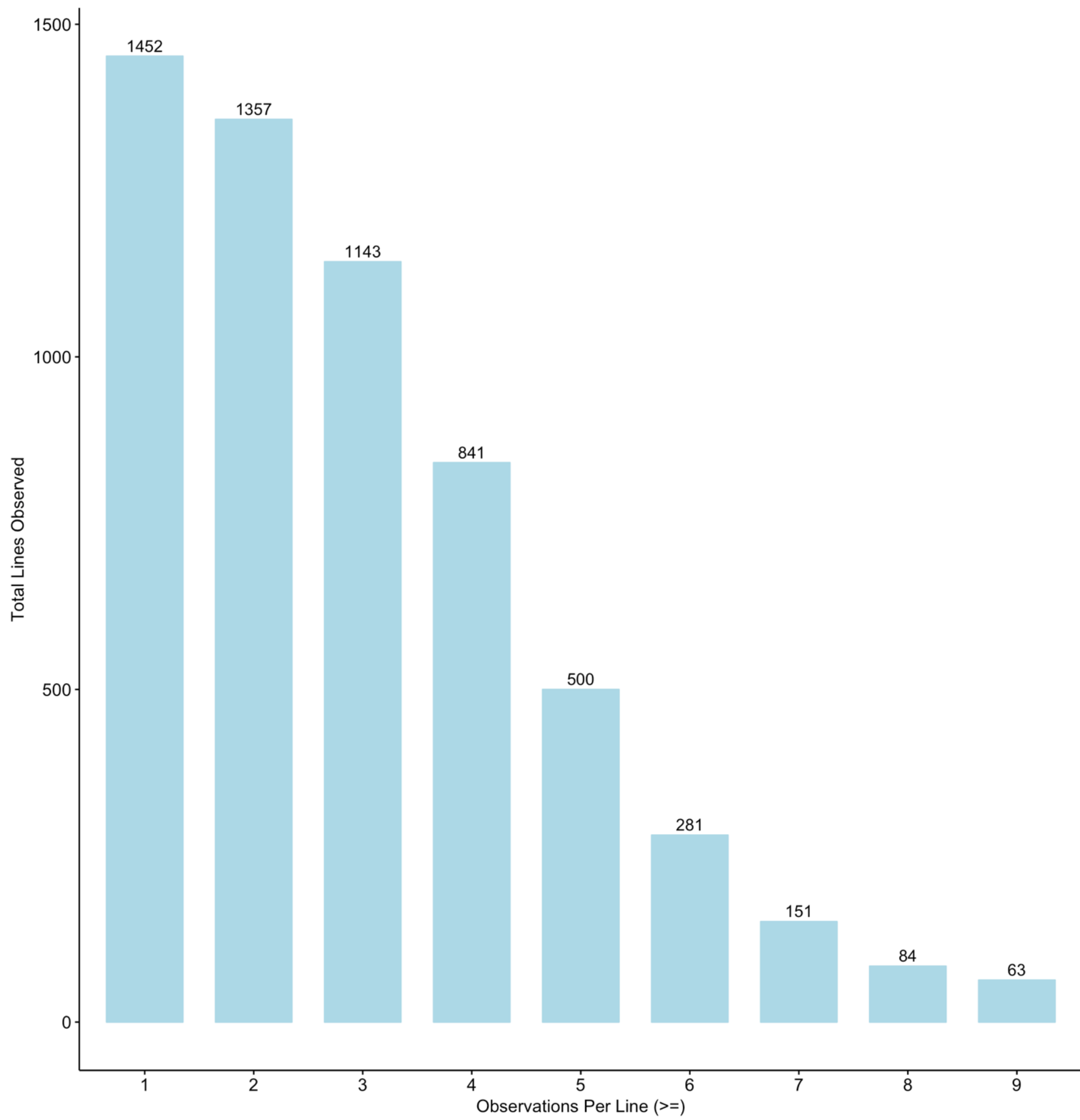

Figure 2.4**.** The distribution of the number of plant introductions and commercial line observations from all tests by the least number of observations per line.

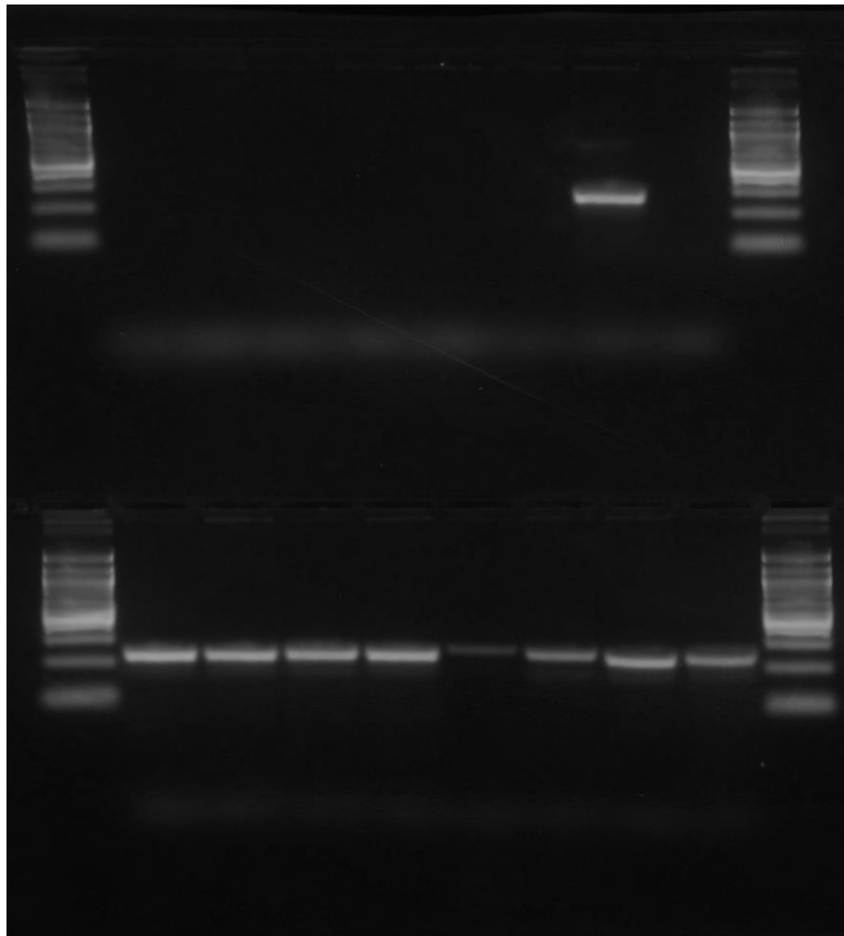

Figure 2.5**.** Differentiation of *Acidovorax citrulli* field isolates into groups I and II by polymerase chain reaction using G2AcFwd/G12AcRev group II-specific (top) and G12AcFwd/G12AcRev Group I- and II-specific (Bottom) primers. Lanes 1-4 are isolates collected from watermelon fields at the Horticultural Crops Research Station at Clinton, NC in 2016; lanes 5 and 6 are isolates collected from watermelon fields at the Horticultural Crops Research Station at Clinton, NC in 2017; lane 7 is ACC00-1 (group II control); and lane 8 is AAC200-30 (group I control).

```
KF944711.1     1  CCGAAGAGATAACACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_5622        1  CCGAAGAGATAA-ACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_5533        1  CCGAAGAGATAA-ACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_5798        1  CG-AAGAGATAA-ACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_5553        1  CCGAAGAGATAACACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_12838       1  CCGAAGAGATAACACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT
AC_12760       1  CCGAAGAGATAA-ACTGCATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGT

KF944711.1    56  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_5622       55  TCCGCCCACGGA-ACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_5533       55  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_5798       54  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_5553       56  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_12838      56  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA
AC_12760      55  TCCGCCCACGGAAACTCCGGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAA

KF944711.1   111  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_5622      109  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_5533      110  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_5798      109  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_5553      111  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_12838     111  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG
AC_12760     110  ATGGAGAATTCGGGTTTCTGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCG

KF944711.1   166  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_5622      164  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_5533      165  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_5798      164  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_5553      166  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_12838     166  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG
AC_12760     165  CGAAGCACTGAACCGTGCGGAAAACACTCCTTCGGACCTGCGGGCATATGCCTAG

KF944711.1   221  GCCGCATTGACAGCAGCAAAAATCG-GCAGTACGT
AC_5622      219  GCCGCATTGACAGCAGCAAAAATCA-GCAGTACGT
AC_5533      220  GCCGCATTGACAGCAGCAAAAATCGAGCAGTACGT
AC_5798      219  GCCGCATTGACAGCAGCAAAAATCGGGCAGTACGT
AC_5553      221  GCCGCATTGACAGCAGCAAAAATCG-GCAGTACGT
AC_12838     221  GCCGCATTGACAGCAGCAAAAATCG-GCAGTACGT
AC_12760     220  GCCGCATTGACAGCAGCAAAAATCG-GCAGTACGT
```

Figure 2.6. Sequence homology (>99%) between plus/plus strands of G12AcFwd (yellow) and G12AcRev (blue) primer derived amplicons of the Clinton field isolates and the partial *Acidovorax citrulli* gene *Aave_2166* (GenBank: KF944711.1).

```
CP000512.1    1  CGATAGGGTTGGGTTCAAGCAGGAGCAGTCGGCACGGCCGAAGAGATAACACTGC
AC_001        1  CGATAGGGTTG-GTTCAAGCAGGAGCAGTCGGCACGGCCGA-GAGAT-ACACTGC

CP000512.1   56  ATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGTTCCGCCCACGGAAACTCC
AC_001       53  ATCCGGACGAGGATCCACGTCTGCAGGATCGTCGAGTTCCGCCCACGGAAACTCC

CP000512.1  111  GGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAAGTGGAGAATTCGGGTTTC
AC_001      108  GGAGCACTCTCCGGCCTGTCGTCAAGAACTGCTGGAAGTGGAGAATTCGGGTTTC

CP000512.1  166  TGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCGCGAAGCACTGAACCGTGC
AC_001      163  TGCCAGAGCGTCCCCGTCAGAAGGCAATAGCTTTGCGCGAAGCACTGAACCGTGC

CP000512.1  221  GGAAAACACTCCTTCGGACCTGCGGGCATATGCCGAGGCCGCATTGACAGCAGCA
AC_001      218  GGAAAACACTCCTTCGGACCTGCGGGCATATGCCGAGGCCGCATTGACAGCAGCA

CP000512.1  276  AAAATCGGCAGTACGT
AC_001      273  AAAATCGGCAGTACGT
```

Figure 2.7. Sequence homology (>99%) between plus/plus strands of G2AcFwd (green) and G12AcRev (blue) primer derived AAC00-1 isolate amplicon and the partial *Acidovorax citrulli* gene *Aave_2166* (GenBank: CP000512.1). The G12AcFwd annealing location is shown in yellow.

Chapter 3

Evaluation of Methods for Testing Resistance to Bacterial Fruit Blotch in Watermelon Fruit

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ABSTRACT

Bacterial fruit blotch, caused by *Acidovorax citrulli*, is a major disease that causes significant damage to the watermelon fields every year. Despite significant efforts to identify foliar resistance to bacterial fruit blotch, no commercial resistance lines are available. From 2015-2017, we evaluated fruit inoculation methods for use in largescale field resistance screenings: (1) immature fruit spray inoculation under wounded vs unwounded and bagged vs unbagged conditions; (2) mature fruit injection inoculations. Inoculum concentrations ranged from 10^3 to 10^8 CFU/ml. During the course of the three-study, treatments conditions changed as we introduced new methods. In 2015, because we inoculated at or shortly after anthesis and wounded fruit, excessive fruit senescence precluded formal analysis. In 2016, we removed the wounding treatment and spray inoculated 1-2-week-old fruit of Charleston Gray, Mickylee, and Crimson Sweet using a bagged vs unbagged over 10^5, 10^6, 10^7, 10^8 CFU/ml concentrations. We found that bagged treatments produced higher disease symptoms but also caused damage that confounded the results. In 2017, there were no significant differences among cultigens or concentrations. In 2017, we removed the bagging treatment, used a wider inoculum concentration range: 10^3, 10^5, 10^7, 10^8 CFU/ml, introduced a mature fruit injection-inoculation method, used Charleston Gray and Sugar Baby fruit, and spray inoculated 1- to 2-week-old fruit. Both the injection and spray inoculation methods separated the two cultigens, but results were inverted: Charleston Gray was more susceptible than Sugar baby in spray inoculations but more

resistance in injection inoculation methods. For spray inoculations, all concentrations performed the same, despite our initial assumptions that a linear relationship would exist. Similarly, the injection-inoculation methods had the same results for the three highest inoculum concentrations, but lowest concentration, 10^3 CFU/ml, was the same severity as the water control, suggesting a cut-off inoculation level. Of the all the methods tested, we conclude that spray inoculation method targeting 1-to 2-week-old fruit at any concentration tested was the best in terms of ease of use, cultigen resistance separation, and large-scale screening amenability.

1 INTRODUCTION

Watermelon *Citrullus lanatus* var. *lanatus* [Thunb.] Matsum. & Nakai is a valuable crop grown on over 8.5 million acres around the world in 2012 (Food and Agriculture Organization of the United Nations, 2014). In 2015, the US harvested 47,125 ha of watermelon at a total value of over $488 million dollars (USDA National Agricultural Statistics Service, 2017). Watermelon is part of the Cucurbitaceae family which includes many important crops such as melon (*Cucumis melo* L.), squash (*Cucurbita pepo* L.), and cucumber (*Cucumis sativus* L.).

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* is a major seed-borne disease affects primarily watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) seedling and fruit production around the world (Latin and Hopkins, 1995, Schaad et al., 2003). The BFB causal agent was first isolated in 1965 from two watermelon plant introductions (PIs) (Webb and Goth, 1965) and, after observing water-soaked lesions on watermelon PIs at the Regional Plant Introduction Station, Experiment, GA, it was further described and classified by Schaad et al. (1978). In 2008, it was considered a separate species and given the current nomenclature: *Acidovorax citrulli* (Schaad et al., 2008). The first report of confirmed BFB in commercial watermelon fields was in the Mariana Islands in 1987 where it was coined "fruit blotch" (Wall

and Santos, 1988). In 1989, it was found in watermelon fields in the United States (Hopkins, 1989); the initial outbreaks in South Carolina and Florida caused watermelon losses nearing 80% (Hopkins et al., 1993). BFB has since spread to most watermelon producing areas of the United States (Somodi et al., 1991, Wall et al., 1990, Hamm et al., 1997). Outbreaks of BFB can lead to a complete loss of production fields, and can cause serious damage, 5% to 50%, to nearby by fields through secondary outbreaks (Latin and Hopkins, 1995).

A. citrulli can infect all growth stages of the watermelon plant: seeds, seedlings, foliage, flowers, and fruit (Latin and Hopkins, 1995). Symptoms of seedling infection are water-soaked, progressing to brown, lesions on the cotyledons and hypocotyl which often quickly leads to plant death (Latin and Hopkins, 1995). Leaf lesions are often discreet, "small, dark brown and somewhat angular" (Latin and Hopkins, 1995) and tend to present along the major leaf veins (Hopkins et al., 1993). There is no evidence of systemic migration of *A. citrulli* in watermelon plants (Rane and Latin, 1992). Infected plants are generally not defoliated, but rather leaf tissue becomes a reservoir for *A. citrulli* that later spread to developing fruit (Bahar et al., 2009, Latin and Hopkins, 1995, Hopkins and Thompson, 2002, Frankle et al., 1993). Watermelon fruit are most vulnerable to *A. citrulli* bacterium entering through stomata during the first two weeks post anthesis, prior to waxy cuticle stomatal plugging (Frankle et al., 1993). Fruit symptoms quickly progress from inconspicuous water-soaked lesions with irregular margins to expanded dark-green lesions that fissure, causing massive internal infection by secondary organisms, which leads to the destruction of the fruit (Latin and Hopkins, 1995, Hopkins et al., 1993). Seeds are then internally and externally infested with pathogen (Rane and Latin, 1992), completing the disease cycle. Infestation can occur in symptomatic fruit or asymptomatic fruit exposed to the pathogen (Hopkins et al., 1996, Carvalho et al., 2013). Seed contamination does not appear to be

affected by storage under dry conditions over 12 months and would likely not be eliminated by longer-term storage (Hopkins et al., 1996). Insidious seed infestation continues to spread the pathogen around the world, representing a serious threat to the watermelon industry.

Currently, the most effective control measure is the careful exclusion of infected plants at seedling production facilities and, ultimately, fields (Hopkins and Thompson, 2002, Hopkins et al., 1996). To that end, research has focused on enhancing BFB detection through molecular techniques (Walcott and Gitaitis, 2000, Walcott et al., 2006, Bahar et al., 2008, Ha et al., 2009). The USDA National Seed Health System gives guidelines and procedures for three detection methods: grow-out assay, seedling PCR, and ISHI Method (seed-based detection) (USDA National Seed Health System, 2017) The standard detection technique is a the seedling grow-out assay where 10,000 to 30,000 seeds per lot are grown for three weeks and compared to positive controls to determine infection (USDA National Seed Health System, 2017). That method adds significant cost to seed production. Another control strategy is to limit the spread of the pathogen during transplant production through the use copper-based bactericides and peroxyacetic acid (Hopkins, 1995, Hopkins et al., 2009). In the field, moderate chemical control can be achieved through the repeated application of copper containing products during early fruit development (Hopkins, 1991, Hopkins et al., 2009). Seed treatments to eliminate the pathogen have also been extensively explored with promising effectiveness (Hopkins et al., 1996, Sowell and Schaad, 1979, Wall, 1989, Hopkins et al., 2003, Feng et al., 2009, Rane and Latin, 1992). However, in some cases, there can be marked decreased post-treatment germination (Feng et al., 2009). However, despite detection efforts and control measures, sporadic BFB outbreaks continue to cause significant damage to watermelon production (Hopkins et al., 2009).

BFB resistance would be a highly valuable in reducing current costly preventative measures and could be become an important part of integrated pest management (IPM). To that end, there have been many laboratory, greenhouse, and field screenings evaluating foliar resistance in watermelon (Hopkins et al., 1993, Hopkins and Thompson, 2002, Carvalho et al., 2013, Ma and Wehner, 2015, Hopkins and Levi, 2008, Sowell and Schaad, 1979) and melon (Bahar et al., 2009, Wechter et al., 2011). During that same period, breeding efforts have shown that foliar resistance is polygenic (Hopkins and Levi, 2008). Despite the intensive efforts by many researchers, there are no resistant commercial watermelon lines available (Johnson et al., 2011). In addition to resistance screenings, an immense body of research has evaluated isolates and lines for pathogenicity, disease incidence, seed treatment efficacy, seed colonization pathways, detection method development, and seed transmission, using a variety of inoculation methods (Table 3.1). Most of the inoculation methods have focused on foliar application and have generally used a narrow inoculum range 10^5 to 10^8 CFU/ml. Among fruit inoculations, as far as investigated, Hopkins and Thompson (2002a) was the only study to evaluate fruit symptoms using a spray inoculation assay. In a separate study that same year, Hopkins and Thompson (2002b) referenced field observations that fruit from foliar resistant lines did not have symptoms of blotch, suggesting that some lines may also have genes for fruit resistance. Although fruit resistance would be valuable for producing disease-free seed and marketable fruit in spite of foliar infection, the lack of research on fruit resistance in favor of foliar screening methods has three probably explanations: 1- large-scale screening at the fruit stage is resource-intensive (Hopkins and Thompson, 2002); 2- foliar resistance may correlate with fruit resistance and, even with susceptible fruit, it may be sufficient for disease control (Hopkins and Thompson, 2002); 3- fruit inoculation methods have not been well established. As far as investigated, there

have not been any significant fruit inoculation screenings nor inoculation methodology evaluations conducted. Fruit resistance may be an additional source of resistance to BFB. An optimized fruit resistance assay would allow researchers to efficiently conduct large scale screenings and breed for fruit resistance.

In this study, we conducted field trials to evaluate inoculation methods obtained from the literature: bagging, wounding, spray inoculation, and fruit injection. Of course, inoculation methods that penetrate the fruit surface, such as wounding and injection assays, may give different results than surface spray inoculations. We were also interested in determining whether there was a linear relationship of inoculum concentration with disease severity. Our first concern was to develop methods that would be effective in field screening for resistance in the fruit. The objectives of this study were to 1- develop an optimized field fruit inoculation method; 2- identify commercial cultivars with variable fruit resistance to be used as checks in other studies; 3- determine the relationship between fruit disease severity and inoculum concentration.

2 MATERIALS AND METHODS

2.1 Inoculum Preparation, Growing Conditions, and Experimental Design

Four group I and one group II isolates recovered from the Horticultural Crops Research Station at Clinton, NC 2015 and stored at -80°C were grown on nutrient agar (NA) (N9405 Nutrient Agar; Sigma-Aldrich, St. Louis, MO) for 48hrs at 30°C. The morning of inoculations, bacterial suspensions were created by rinsing *A. citrulli* colonies from the NA plates with sterilized dH_2O. The bacterial suspensions from each isolate were diluted with sterilized dH_2O to an OD600 of 0.25 [approximately 10^8 colony forming units (CFU)/ml] using a spectrophotometer (UV160U; Shimadzu, Columbia, MD). Calibrated isolates were combined in equal portions and serially diluted with sterilized dH_2O to create the concentrations used in this

study. Water controls were created by rinsing new NA plates with dH_2O. Inoculum and water controls were stored at shaded ambient field temperatures (~30°C) immediately prior to use. Inoculations started as soon as developing were observed in the field.

In the spring, watermelon cultivars were direct seeded (2015 and 2017) or transplanted as five-week-old seedlings from the greenhouse (2016) into raised beds covered with black plastic mulch at the Horticultural Crops Research Stations at Clinton, North Carolina. Rows were 61 m long on 3.1 m centers with 1.2 m spacing between plots and 0.6m spacing between plants within plots. In 2015 and 2016, six-plant-plots were used and, in 2017, three-plant-plots were used. Plants were spiral trained once at just prior to anthesis. The soil at the Horticultural Crops Research Station at Clinton is slightly acidic and sandy with little organic matter and receives ~121 cm annual rainfall with the majority occurring June through August (NCDA&CS, 2017). Fertilizer and water were provided via drip irrigation tubes under the plastic. Prior to planting, the fields were prepared with 10-8.3-4.4 (N-P-K) fertilizer at a rate of 561 kg ha^{-1}. After planting, fertilizer was regularly applied at 224 kg ha^{-1} of 13.5-0-19.8 (N-P-K) and 112 kg ha^{-1} of calcium and 15.5-0-0 9 (N-P-K). Pesticides were used as needed for insect control in accordance with best practices for the area.

Each year a factorial design was used to compare inoculation methods. Over the course of three seasons of testing, various inoculation methods were evaluated on commercial cultigens with putative foliar resistance: inoculum concentration, fruit age at inoculation, bagged vs. unbagged, damaged vs undamaged, mature fruit injection, and spray inoculation. New methods and adjustments were added in the 2016 and 2017 as the experiment developed.

2.2 2015 Inoculation Methods

In order to identify the optimum inoculation method, we evaluated 'Crimson Sweet' (CS) (Willhite Seed Inc., Poolville, Texas), 'Mickylee' (ML) (Hollar Seeds, Rocky Ford, CO), and 'Charleston Gray' (CG) (Sakata Seed America, Morgan Hill, CA) under several treatment combinations: two inoculum concentrations, damaged vs. undamaged, bagged vs. unbagged, and two fruit age markers for inoculation timing. In all cases, fruit were sprayed to run off with inoculum and, in order to decrease the possibility of escapes, resprayed 3 to 4 days later. Inoculum was prepared as previously described and calibrated to two concentrations: (high) 10^8 CFU/ml and (low) 10^6 CFU/ml. Just prior to inoculation, fruit in the damage treatment were pricked using a sterile toothpick. Immediately after spray inoculation, fruit were either bagged for 72 hrs using a quart-sized Ziploc bag (S. C. Johnson and Son, Inc., Racine, WI) or left unbagged. Inoculations were timed to occur just after anthesis when the flower had closed and was still yellow or just as the flower had turned brown and had begun to separate from the developing fruit. Fruit inoculations started as soon as developing fruit was observed in the field and occurred over three inoculations dates at one week intervals to capture as many fruit as possible for analysis. Disease severity was rated 21 days post inoculation using a 0 to 9 scale based on the percentage of the upper fruit surface showing symptoms. Fruit within each plot were averaged for analysis. There were 210 test plots representing 192 treatment combinations and 18 water control plots. Each treatment combination was replicated four times.

2.3 2016 Inoculation Methods

Based on our experience in 2015, we used the same cultivars, ML, CS, and CG, and bagged fruit and spray inoculated as we did in our 2015 methods trial, but we increased the range of inoculum concentrations: 10^5, 10^6, 10^7, and 10^8 CFU/ml, eliminated the damage treatment, and

delayed inoculations until 1-2 weeks post anthesis. Exact timing of fruit in the field was not feasible, so physiological immaturity markers typical of early maturing fruit 1-2 weeks post anthesis (Daley and Wehner, unpublished observations) were used to gauge fruit readiness: fruit size generally ranging 5 to 13 cm in diameter and, most importantly, a glossy, soft wax cuticle. Fruit spray inoculations occurred over three inoculation dates, and disease ratings proceeded as described above. Each treatment combination was replicated four times across 96 plots. The water control fruits were randomly selected from among each treatment combination that presented multiple fruit at the same age.

2.4 2017 Inoculation Methods

In the spring of 2017, using a wider range of concentrations than we used in 2016, we introduced mature watermelon injection-inoculations and, again, spray-inoculated immature watermelon fruit. We tested 96 plots for each inoculation method consisting of alternating CG and SB (Sakata Seed America, Morgan Hill, CA); each with 10 replications per treatment combination and 16 water control plots representing each treatment combination. Inoculum was produced as described above and diluted to create 10^3, 10^5, 10^6, 10^8 CFU/ml concentrations. Spray inoculations proceeded as performed in 2016. For fruit injections, we utilized the method described by Burdman et al. (2005) with slight variations. Briefly, mature watermelons were identified by the die-back of the primary tendril, and at least three watermelon fruit per plot were injected 1 cm deep with 1 ml of bacterial suspension at each of two equidistant locations along the length of the fruit using a 0.45 mm needle (3ml Sub-Q Syringe with Luer-Lok Tip with BD PrecisionGlide Needle; BD Becton, Dickinson and Co., Franklin Lakes, NJ). Sterilized water-inject fruits were used as negative controls.

Two inoculation dates for spray inoculations and a single injection-inoculation date were used to identify a maximum number of immature and mature fruit, respectively. Disease severity for both methods was evaluated 21 days post inoculation. Spray inoculations were rated as described above and injection-inoculations were rated using the scale described by Burdman et al. (2005) and Walcott et al. (2004): 0, no symptoms; 1, small rind surface lesions; 2, large lesions penetrating into the rind; 3, large lesions penetrating into the fruit; 4, partial collapse of the fruit tissue; 5, complete fruit rot.

2.5 Statistical Analysis

Statistical analyses were performed using the R environment (R Development Core Team, 2017). The 2016 and 2017 data were run through type II analysis of variance using the 'car' 2.1-3 package (Mendiburu, 2016) *Anova()* function to compare the plot disease severity for each cultigen, inoculum treatment, and interaction. Each year was independently analyzed, and the 2017 injection-inoculation data was analyzed separately from the 2017 spray-inoculation data. A Post-hoc Tukey HSD test was conducted using the 'lsmeans' 2.26-3 package (Lenth, 2016) *lsmeans()* and *cld()* functions to compare injection-inoculated inoculum concentrations ($p < 0.05$). Data were visualized using the 'ggplot2' 2.2.1 package (Wickham, 2009) *ggplot()* function.

3 RESULTS

3.1 2015 Methods Screening Observations

No analysis was conducted in 2015 because of excessive fruit senescence. However, there were critical observations that facilitated the screening of the *Citrullus* spp. germplasm collection in 2015 to 2017 (Chapter 2) and further methods testing in 2016 and 2017. Inoculum spray, regardless of the concentration test, too early in fruit development led to high rate of

senescence. During the 3rd and 4th inoculations it was apparent that the 1st and 2nd ratings were senescing at a high rate; suspecting that inoculations were occurring too early in development, during the 3rd and 4th inoculations slightly older fruit within the 1-2-week range were selected. This corresponds with previous reporting that the most vulnerable time for fruit infection up to two weeks post inoculation (Frankle et al., 1993). We observed that allowing fruit to develop at least one week to decreased the abortion rate. These older fruits produced disease symptoms that gradually increased over time and were easy to rate at 21 dpi. However, by the 3rd and 4th inoculations, there were too few fruit to analyze. The 2015 methods test provided the foundation for subsequent testing, providing us with ideas for a practical inoculation method.

3.2 2016 Methods Screening Results

Factorial Analysis of variance of all main effects and interactions was significant for the bagged vs. unbagged main effect ($F[1,38] = 20.36$, $p < 0.001$) with disease severity (0 to 9) LSmeans of 4.4 (SEM= 0.41) and 1.9 (SEM= 0.40), respectively (Table 3.2; Figure 3.1) and the three-way interaction ($F[6,38] = 2.43$, $p = 0.043$). However, field observations suggested that the observed increase in disease severity by bagging was likely confounded by a high incidence of damage exacerbated by the bagging conditions (likely temperature and high humidity) which often led to fruit wilting and browning. These results often mimicked blotch symptoms and caused early senescence. Despite literature suggesting variability in resistance, we did not detect differences between the cultigens tested: CG, CS, and ML. The three-way interaction was significant because of an unexplained higher disease severity for bagged CG at the 10^6 CFU/ml inoculum concentration.

3.3 2017 Methods Screening Results

Among the spray-inoculated fruit, all inoculum concentrations produced statistically indistinguishable results, and cultigen was the only significant effect for disease severity ($F[1,55] = 12.65$, $p < 0.001$) with no significant interaction with concentration (Table 3.3). Cultigens, over all concentrations combined, indicated that the SB LSmean (1.4, SEM = 0.42) was significantly different from the CG mean (3.5, SEM = 0.44) (Figure 3.2), matching expectations for these cultivars based on our prior observations. However, cultigens analyzed at each concentration were not significantly different at the $p = 0.05$ level. Spray inoculated water controls were asymptomatic and were excluded from the statistical analysis.

Injection-inoculated watermelons showed no symptoms, significant blotching around the injection sites, or split perfectly along the length of the fruit as if the fruit had been sliced open. The length-wise split occurred suddenly as the fruit bursts open, ejecting the rotting flesh. In addition to the high incidence of splitting, the injection-inoculated fruit exhibited a differences compared to the spray inoculation results. First, water controls produced significant damage to the fruit, likely caused by exogenous surface pathogens being either introduced into the fruit at the time of inoculation or later through movement into the wound. Second, post-hoc Tukey HSD tests of the inoculum concentrations and water control showed that the lowest concentration tested, 10^3 CFU/ml, produced disease severity similar to the water control and significantly different from the other concentrations (Figure 3.5). All other concentration comparisons were not significantly different from each other. Third, cultigen across all inoculation concentrations combined was significant in the opposite direction ($F[1,82] = 8.05$, $p < 0.01$), with CG having a lower LSmean than SB, 3.3 (SEM = 0.17) and 4.1 (SEM = 0.18), respectively (Table 3.4).

Cultigen was not significant at individual concentrations nor was there a significant cultigen by concentration interaction.

4 DISCUSSION

4.1 Fruit Inoculation Method Development

The objective of this study was to determine an effective watermelon fruit inoculation method. To that end, we considered the various fruit inoculation methods presented in the literature (Appendix A). The spray inoculation method was based on Hopkins et al. (1993), Hopkins et al. (1996), Hopkins et al. (2002a), Walcott et al. (2004), and Carvalho et al. (2013). The injection method was based on Burdman et al. (2005). The bagging method was based on Rane and Latin (1992), Dutta et al. (2008), and Walcott et al. (2004). There was no precedent in the literature for fruit wounding, but Yan et al. (2017) has since demonstrated a successful pathogenicity test using a wounding technique on detached immature melon fruit. Although we generated our own 0 to 9 severity scale based on surface percentage damaged for spray inoculations, we evaluated injection-inoculated fruit using a 0 to 5 scale adapted from Walcott et al. (2004) and Burdman et al. (2005). It is important to note that all of the prior fruit assays were used for isolate pathogenicity and/or disease incidence studies and not specifically for resistance screening. However, we hypothesized that variable isolate pathogenicity and aggressiveness was evidence that variable resistance may also be observable between different hosts. Our data showed that the spray-inoculation method was the most reproducible and differentiated the cultigens according to our expectations. Our spray method was most similar to that of Hopkins et al. (1993), except that we used younger fruit (<14 dpa) and rated for disease severity. Nevertheless, given the strong environmental effect on BFB (Hopkins and Thompson, 2002) and the genetic variation among *A. citrulli* isolates (Walcott et al., 2000, Walcott et al., 2004,

Burdman et al., 2005, Eckshtain-Levi et al., 2014), our results should be taken in context of the environmental conditions and isolates used.

This research was the first major attempt to optimize a method for *A. citrulli* watermelon fruit inoculation for resistance screening. Although, further testing would be required, these methods may be extendable to other cucurbits.

4.2 Environmental Considerations

Spray inoculation mimics natural infection, but is variable under field conditions, even on the inbred cultivars tested, requiring high replication to distinguish differences. Our observed high variability was similar to field observations by Hopkins et al. (1993) in which no significant differences among cultivars in the field could be detected. However, in that same study, significant differences were detected when seedlings of the same cultivars were tested under greenhouse conditions. Moreover, in our study, weekly inoculation dates were used during fruit set in order to include as many fruit as possible. Ultimately, data from these inoculation dates were combined for analysis, adding variation due to inoculation date.

Furthermore, we found that inoculation timing was important for producing consistent results. Our decision to inoculate at anthesis in 2015 and bag fruit in 2015 and 2016 was based on a greenhouse method described by Dutta et al. (2012) to evaluate seed infestation rates. In that study, the flower ovary was swabbed with ~10^6 CFU/ml inoculum followed by bagging for 24 hrs. Walcott et al. (2004) rated disease severity of inoculated greenhouse watermelon and melon fruit at 3 to 9 days-post-anthesis. However, in 2015, we had high rates of senescence contrary to the fruit development observed in a similar greenhouse study (Dutta et al., 2012). This discrepancy is likely an unexplained effect of greenhouse-based assays vs. field assays. Indeed, our observations were analogous to the frequent field abortions observed by O'Brien and Martin

(1999) in naturally infected melon “fruitlets” in the field. We found that targeting 1-2-week-old fruit resolved the senescence issue and produced assessable symptoms. Our results corresponded to observations by O’Brien and Martin (1999) that when infection occurred in a more mature melon fruit, the fruit did not abort and the disease proceeded normally. In order to identify properly aged fruit, we had to rely on fruit size and waxy cuticle to identify fruit within 1-2 weeks post-anthesis. Whereas anthesis was a discreet biological marker for fruit age, the inexact timing of our method undoubtedly introduced variation, but proved to be practical for large-scale field inoculations. It is likely that improved methods that standardize fruit age would decrease error variance.

Bagging was attempted in order to increase humidity as reported in seedling assays (Eckshtain-Levi et al., 2014) and fruit assays (Dutta et al., 2012, Walcott et al., 2004, Rane and Latin, 1992). However, in our 2015 and 2016 studies, we found that bagging increased disease severity, confounded disease symptoms, and reduced differences among cultigens. Similarly, Eckshtain-Levi et al. (2014) reported that plants had to be excluded from the study because of sunscald damage induced by bagging. We did not include bagging as a treatment in 2017 tests because of excessive damage to fruit. The successful use of bagging under field conditions seen by Rane and Latin (1992) and our failure can be partially explained by the methods we used. Because of practicalities of getting to our fields, bags were removed 72hrs after inoculation rather than 24 hrs (Dutta et al., 2012) or 48 hrs (Walcott et al., 2004, Rane and Latin, 1992). It is possible that the extra 24 to 48 hrs of bag time caused increased sunscald. Another consideration is that we did not attempt to control heat while maintaining high humidity as was done by Rane and Latin (1992), under field conditions, by covering the plastic bag with a brown paper bag. In contrast to our observations, Rane and Latin (1992) noted that bagging led to decreased disease

incidence for one of their isolates. Nevertheless, our bagging method did not help distinguish the resistance of cultigens and added significant labor to the assay. Bagging may be more appropriate for studies such as those by Walcott et al. (2004) and Dutta et al. (2012a) rather than field studies.

4.3 Injection vs Spray Inoculation Methods

We hypothesized that an injection assay would differentiate between resistant and susceptible lines based on pathogenicity results from Burdman et al. (2005) that corresponded with group I and group II strains having known aggressiveness. Additionally, a recent assay using a pinprick inoculation on immature melon fruit showed pathogenicity differences between group I and group II isolates (Yan et al., 2017). Water controls in the injection test performed similarly to the lowest inoculum concentration, suggesting that extra unknown microbes present on the needles or fruit surface are likely causing damage. Higher inoculum concentrations all performed the same and had means near the top of the rating scale. In our study, needles were not mixed among the concentrations but were not sterilized between inoculations, so there may have been transmission of pathogens between watermelon fruit. Certainly, injections could have introduced field pathogens from unsterilized fruit surfaces. Injection assays in more controlled and sterile conditions may have lower interference by other pathogens; hence, injection methods may be better suited for lab or greenhouse conditions.

Burdman et al. (2005) cautioned that artificial inoculation methods (particularly injection inoculation) could obscure important differences among isolates (i.e., bacterial motility) that would alter pathogenicity. ‘Sugar Baby’ was relatively resistant compared to ‘Charleston Gray’ in the spray inoculation test, but not in the injection method test; that suggests that fruit surface features may contribute important resistance, such as stomatal plugging observed by Frankle and

Hopkins (1993). In short, fruit are typically infected via open stomata during the first two weeks post anthesis (Frankle et al., 1993), and a wounding assay likely bypasses plant defenses (R. R. Walcott, personal communication, 2015). Hence, a wounded-fruit assay may not be suitable for *A. citrulli* resistance screening, while appropriate for pathogenicity assays.

There was a significant difference between the two cultivars in the injection-inoculation method that may have been caused by size differences between the cultivars. These injection-inoculated results were marginally significant and might be accounted for by the size differences between the two cultivars as injection sites were equidistantly placed on the upper surface of each fruit, resulting in CG having a slightly greater distance between the injection sites. The greater distance could limit interaction between infection sites, decreasing severity on large CG fruit relative to medium-sized SB fruit. Alternatively, CG could possess greater internal resistance than SB, suggesting another mechanism that could be exploited.

For both inoculation methods, one of the goals was to determine a relationship between inoculum concentration and disease severity. We expected to observe a linear relationship; however, inoculum concentration differences were not significant in either the 2016 or 2017 spray inoculation tests and only significant at the lowest concentration (matching dH_2O levels) in the injection methods test. While the lowest concentration we used was ~10^3 CFU/ml and the lowest reported concentration is 8 x 10^1 CFU/ml (Tian et al., 2016), there is no literature quantifying the concentration of natural inoculum splashed from leaf tissue to developing fruit, and it is likely that all studies have used grossly concentrated inoculum concentrations. Additional research needs to be conducted to quantify natural inoculum concentrations and identify the absolute minimum inoculum concentration required for symptom development.

4.4 Observed Cultivar Resistance Compared to Prior Reporting

The three cultivars we used in 2016, ML, CS, and CG, had different levels of disease resistance in foliar assays (Hopkins et al., 1993, Hopkins and Thompson, 2002). However, we were unable to differentiate the three cultivars in our tests. For our spray and injection inoculation methods testing in 2017, we used CG and SB, with reported varied disease incidence (Carvalho et al., 2013, Hopkins et al., 1993) and different fruit resistance (unpublished data, Daley and Wehner, 2016). Our failure to separate cultigen means in 2016 has two possible explanations. First, there may have been a flaw in our experiment design or a deficiency in our inoculation method. In 2016, it is possible that wounding and bagging treatments may have confounded our ability to detect differences due to decreased number of observations. In 2017, wounding and bagging treatments were not performed and all inoculum levels produced similar symptoms, maximizing the number of observations for cultivar comparisons. A more interesting explanation is that reported foliar resistance does not necessarily correlate with fruit resistance. This phenomenon of resistance being dependent on developmental stage has been reported for watermelon (Carvalho et al., 2013, Hopkins et al., 1993) and melon (Wechter et al., 2011, Bahar et al., 2009). Our failure to separate cultivars in 2016 may be evidence of variable resistance, though more testing would be required. In contrast, Hopkins and Thompson (2002b) observed possible fruit resistance in their foliar resistant lines, suggesting correlation. However, it is generally accepted that one of the major challenges of BFB resistance screening is that resistance is dependent on the developmental stage of the plant and the strains tested (Johnson et al., 2011). The findings presented here, coupled evidence from the literature, suggest that an unexplored resistance may be found at the fruit stage, again highlighting the importance of developing methods for fruit inoculation so that screenings can be effectively conducted.

5 CONCLUSIONS

Among the methods tested, the spray inoculation method on 1-2-week old fruit successfully differentiated cultigens, provided the highest consistency, and was relatively easy to administer. Our spray method most resembles the fruit inoculation method used by Hopkins and Thompson (2002a). Spray inoculations take advantage of natural infection pathways and resistance mechanisms (Frankle et al., 1993). Although we expected to see a linear relationship between inoculum concentration or a 'cut-off' inoculum concentration, for spray inoculations, none of the concentrations were significantly different and, for injection inoculations, only the lowest concentration, 10^3 CFU/ml, produced symptoms equivalent to the water control. The entire range of inoculum concentrations tested can be used for spray inoculations, and all but the lowest concentration can be used for injection inoculations. Because lower concentrations can be used for inoculation, it is easier to generate large volumes of inoculum.

Under our tested conditions, bagging, wounding, and injecting added significant labor and did not improve cultigen differentiation. It is likely that bagging and injecting may be better used for controlled-environment studies such as those by Walcott et al. (2004) and Dutta et al. (2012a) rather than field studies. Our injection assays did not improve differences among cultigens for fruit symptoms and did not correlate with our results from spray inoculation. Injection assays bypass the mechanisms for fruit surface resistance and could provide insight into internal mechanisms, though this needs to be further tested under sterile conditions. As per Burdman et al. (2005), injections may be more appropriate for detecting differences for isolate pathogenicity. Wounding by using the toothpick prick assay under field conditions was not tested beyond field observations in 2015, so firm conclusions cannot be drawn. However, because it has been shown effective for pathogenicity tests (Yan et al., 2017), further testing is warranted.

We suspect that wounding may be better suited for sterile laboratory conditions and would produce results similar to our injection assay.

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Table 3.1. Analysis of variance for bacterial fruit blotch disease severity of spray inoculated immature fruit of three watermelon cultivars at four inoculum concentrations and two bagging conditions in a 2016 field test.

Source	*df*	*MS*	*F*
Cultigen	2	1.23	0.28
Bag/No Bag	1	89.64	20.36***
Concentration	3	9.56	2.17
Cult x Conc	6	5.54	1.26
Bag x Conc	3	3.98	0.90
Cult x Bag	2	10.90	1.95
Cult x Bag x Conc	6	10.71	2.43*
Residuals	38	4.40	

Significance codes: ‘***’ 0.001, ‘**’ 0.01, ‘*’ 0.05

Table 3.2. Analysis of variance for bacterial fruit blotch disease severity of spray inoculated immature fruit of two watermelon cultivars at four inoculum concentrations in a 2017 field test.

Source	*Df*	*MS*	*F*
Cultigen	1	72.72	12.65***
Concentration	3	4.22	0.73
Cult x Conc	3	2.74	0.48
Residuals	55	5.75	

Significance codes: '***' 0.001, '**' 0.01, '*' 0.05

Table 3.3. Analysis of variance for bacterial fruit blotch disease severity of injection inoculated mature fruit of two watermelon cultivars at five inoculum concentrations in a 2017 field test.

Source	*df*	*MS*	*F*
Cultigen	1	11.35	8.05**
Concentration	4	15.17	10.75****
Cult x Conc	4	2.58	1.83
Residuals	82	1.14	

Significance codes: '****' 0.0001, '***' 0.001, '**' 0.01, '*' 0.05

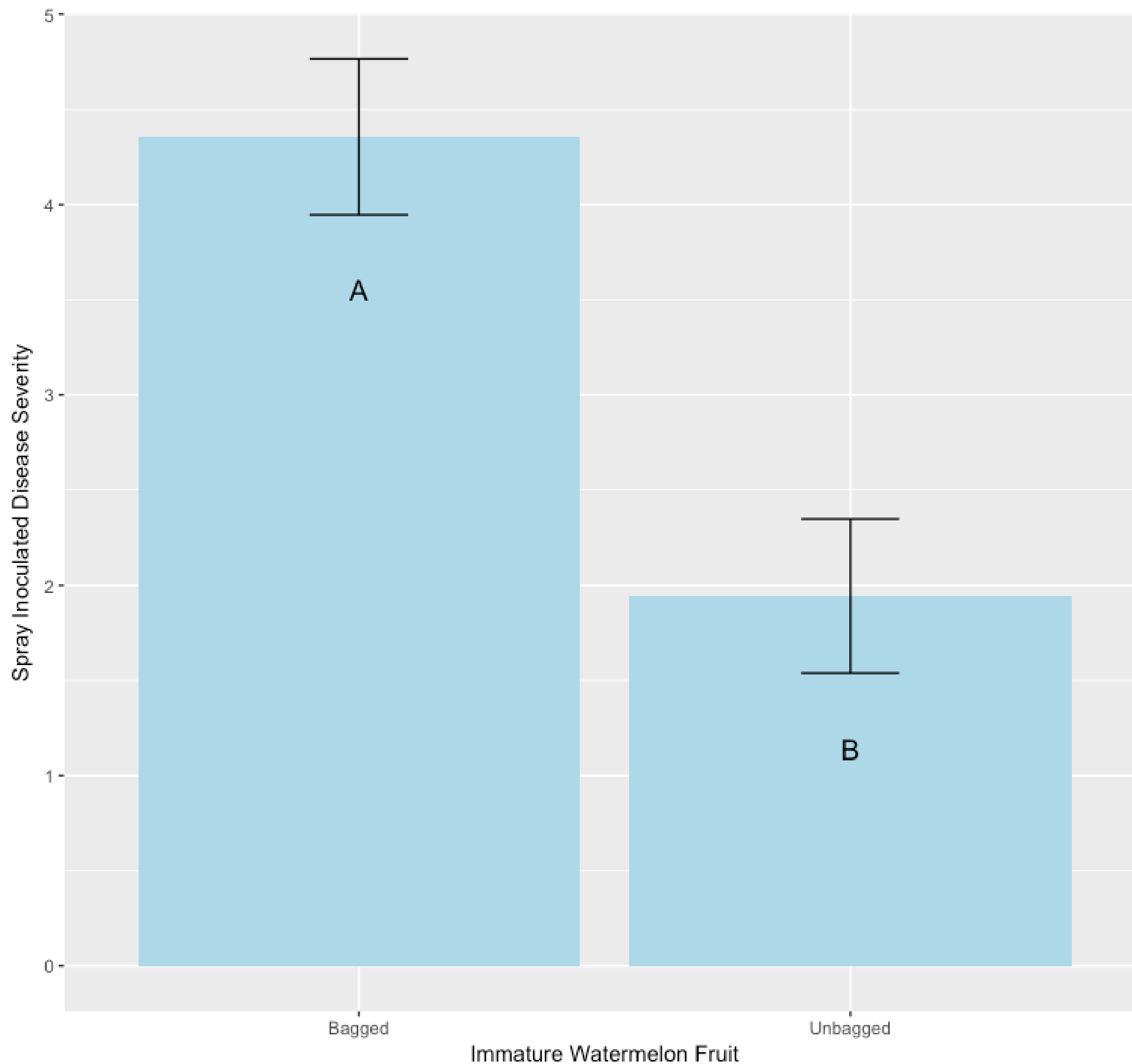

Figure 3.1. Predicted 2016 spray inoculated disease severity (LSmean ± SEM) of bagged (ML [n=11], CS [n=11], and CG [n=13]; LSmean = 4.4, SEM = 0.41) and unbagged (ML [n=11], CS [n=11], and CG [n=8]; LSmean = 1.9; SEM = 0.40) immature watermelon fruit 21 dpi of all inoculum concentrations combined (~10^8 CFU, ML [n=3], CS [n=6] and CG [n=6]; ~10^7 CFU, ML [n=6], CS [n=4] and CG [n=5]; ~10^6 CFU, ML [n=6], CS [n=6] and CG [n=5]; ~10^5 CFU, ML [n=6], CS [n=5] and CG [n=4)] rated on a 0-9 scale corresponding to percent surface symptoms. Letters indicate a significant difference according to the type II ANOVA ($F[1, 38] = 20.36$, $p < 0.0001$).

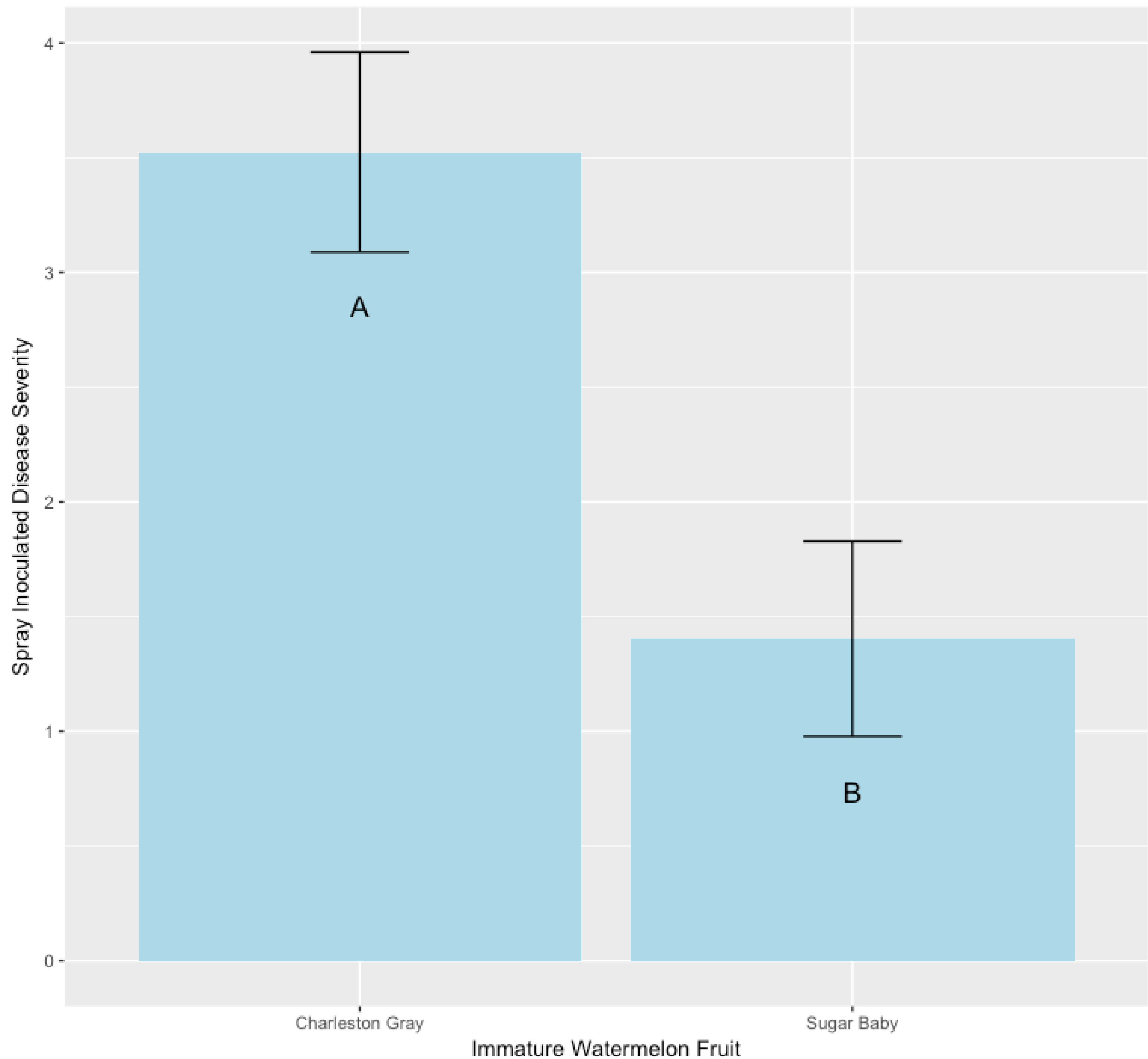

Figure 3.2. Predicted 2017 spray inoculated disease severity (LSmean ± SEM) of immature Charleston Gray (CG) (n = 31, LSmean = 3.5, SEM = 0.44) and Sugar Baby (SB) (n = 32, LSmean = 1.4, SEM = 0.42) fruit 21 dpi of all inoculum concentrations combined (~10^8 CFU, CG [n=6] and SB [n=8]; ~10^6 CFU, CG [n=9] and SB [n=9]; ~10^5 CFU, CG [n=8] and SB [n=7]; ~10^3 CFU, CG [n=8] and SB [n=7] rated on a 0-9 scale corresponding to percent surface symptoms. Letters indicate a significant difference according to letters indicate a significant difference according to the type II ANOVA ($F[1, 55] = 12.65$, $p < 0.001$).

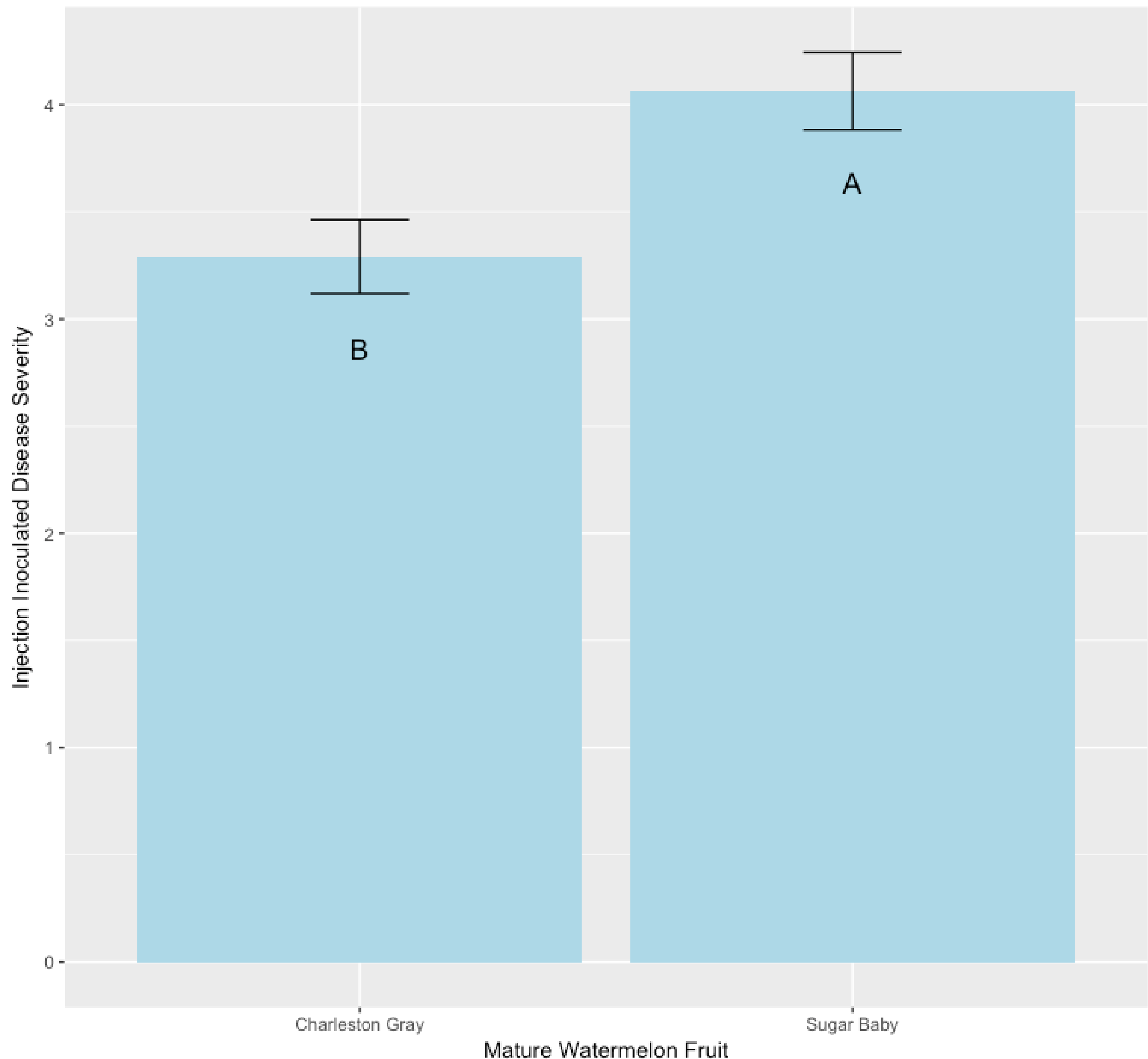

Figure 3.3. Predicted 2017 injection inoculated disease severity (LSmean ± SEM) of immature Charleston Gray (CG) (n = 40, LSmean = 3.3, SEM = 0.17) and Sugar Baby (SB) (n = 37, LSmean = 4.1, SEM = 0.18) fruit 21 dpi of all inoculum concentrations combined (~10^8 CFU, CG [n=10] and SB [n=9]; ~10^6 CFU, CG [n=10] and SB [n=10]; ~10^5 CFU, CG [n=10] and SB [n=10]; ~10^3 CFU, CG [n=10] and SB [n=8] rated on a 0-5 scale. Letters indicate a significant difference according the type II ANOVA ($F[1, 82] = 8.05$, $p < 0.001$)

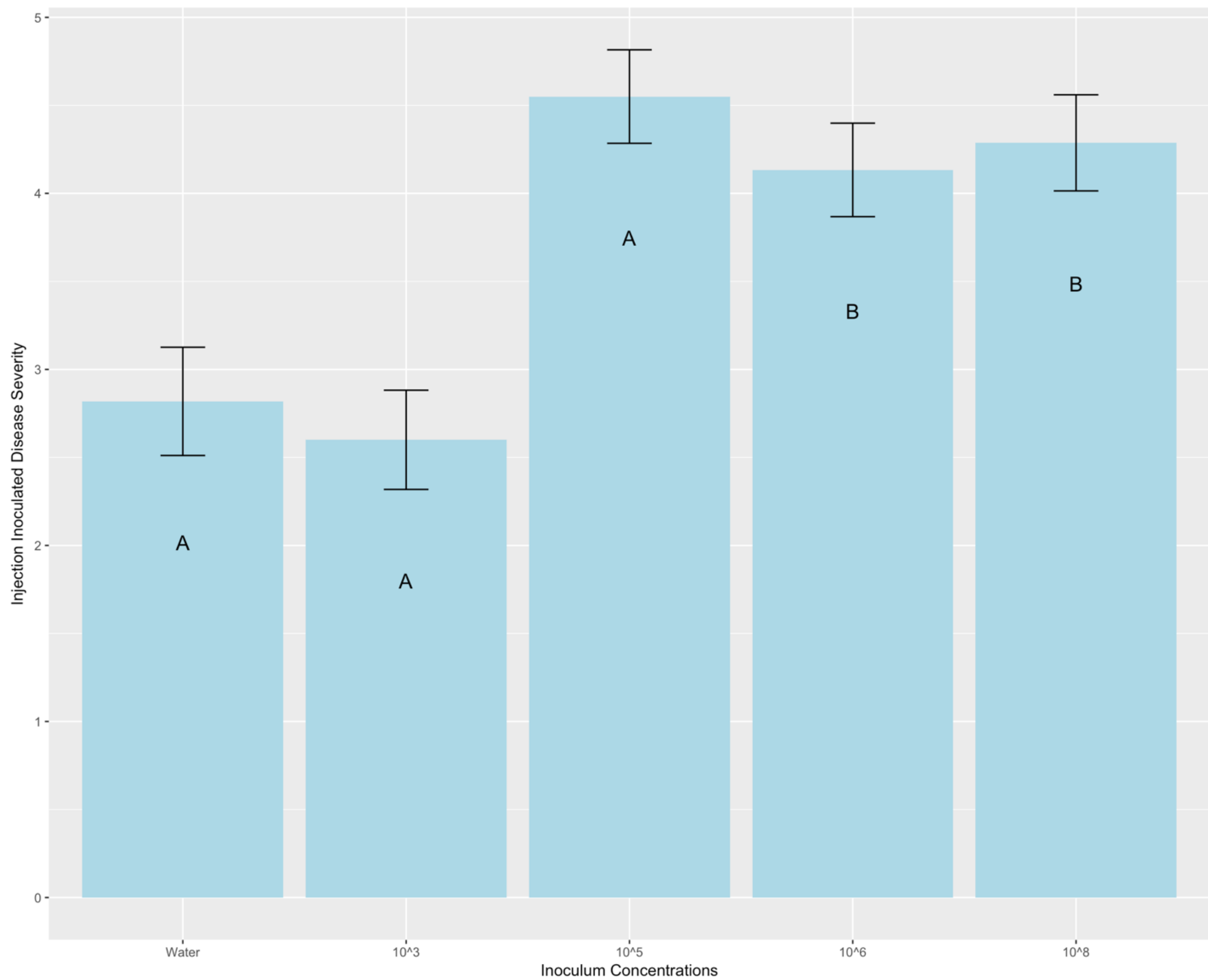

Figure 3.4. Predicted 2017 injection inoculated disease severity (LSmean ± SEM) of immature Charleston Gray (CG) and Sugar Baby (SB) fruit 21 dpi combined across five inoculum concentrations and water: ~10^8 CFU [n=19, LSmean = 4.3, SEM = 0.27]; ~10^6 CFU [n = 20, LSmean = 4.1, SEM = 0.27]; ~10^5 CFU [n = 20, LSmean = 4.6, SEM = 0.27]; ~10^3 CFU [n = 18, LSmean = 2.6, SEM = 0.28]; dH_2O [n = 15, LSmean = 2.8, SEM = 0.31] rated on a 0-5 scale. Letters indicate a significant difference according to Tukey HSD test (p = 0.05).

Chapter 4

Broad-sense Heritability and Genetic Variance Estimates for Resistance to Bacterial Fruit Blotch in Watermelon Fruit

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ABSTRACT

Bacterial fruit blotch (BFB) (Walcott et al., 2004) caused by *Acidovorax citrulli* is a serious threat to watermelon production around the world. Although there have been many BFB resistance studies, heritability has never been estimated. The aim of this study was to calculate broad-sense heritability (H^2) and genetic variance estimates for BFB resistance in watermelon fruit using minimum observation subsets of data from a prior three-year study on *Citrullus* spp. plant introductions in Clinton, NC. Using these estimates, we designed scenarios with varying allocations of years, blocks within year, and replications in order to determine conditions that maximize H^2 over the highest number of lines and least amount of time. The calculated H^2 estimates over the data subsets ranged 0.241 to 0.313 with an average of 0.276, suggesting that breeding will require high-replication testing in advanced generations. We also found that blocks within year was a strong variance source and that year was a minor variance source. In our experimental optimization study, within each set of lines, the highest simulated H^2 estimates were at two years with eight blocks per year, but the highest H^2 per year estimates were at one year with 16 blocks. The highest simulated H^2 was 0.66 in which 250 lines were replicated four times per complete block over two years with eight blocks per year, and the highest simulated H^2 per year was 0.62 in which 250 lines were replicated four times per complete block over one year with 16 blocks per year. The simulated H^2 using 1000 lines replicated once per block over one year had an estimated H^2 of 0.51 and provided the widest testing conditions and a moderately

high H^2estimate. Our results suggest that BFB resistance H^2 is strongly affected by the environment but that it can be improved using optimized breeding designs.

1 INTRODUCTION

Watermelon *Citrullus lanatus* var. *lanatus* [Thunb.] Matsum. & Nakai is a valuable crop grown on over 8.5 million acres around the world in 2012 (Food and Agriculture Organization of the United Nations, 2014). In 2015, the US harvested 47,125 ha of watermelon at a total value of over $488 million dollars (USDA National Agricultural Statistics Service, 2017). Watermelon is part of the Cucurbitaceae family which includes many important crops such as melon (*Cucumis melo* L.), squash (*Cucurbita moschata* L.), and cucumber (*Cucumis sativus* L.).

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* (Schaad et al., 2008) is a major seed-borne disease that primarily affects watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) seedling and fruit production around the world (Latin and Hopkins, 1995, Schaad et al., 2003). The BFB causal agent was first isolated in 1965 at the Regional Plant Introduction Station, Experiment, GA from two watermelon plant introductions (PIs) that presented water-soaked lesions on their leaves (Webb and Goth, 1965). The first report of confirmed BFB in commercial watermelon fields was in the Mariana Islands in 1987 where it was coined “fruit blotch” (Wall and Santos, 1988). In 1989, it was found in watermelon fields in the United States (Hopkins, 1989); the initial outbreaks in South Carolina and Florida caused watermelon losses nearing 80% (Hopkins et al., 1993). BFB has since spread to most watermelon producing areas of the United States (Somodi et al., 1991, Wall et al., 1990, Hamm et al., 1997). Outbreaks of BFB can lead to a complete loss of production fields, and can cause serious damage, 5% to 50%, to nearby fields through secondary outbreaks (Latin and Hopkins, 1995).

Acidovorax citrulli can infect all growth stages of the watermelon plant: seeds, seedlings, foliage, flowers, and fruit (Latin and Hopkins, 1995). Seedling symptoms are water-soaked, brown lesions on the cotyledons and hypocotyl which often leads to plant death (Latin and Hopkins, 1995). Leaf lesions are often discreet, “small, dark brown and somewhat angular” (Latin and Hopkins, 1995) and tend to present along the major leaf veins (Hopkins et al., 1993). Infected plants are generally not defoliated, but rather leaf tissue becomes a reservoir for *A. citrulli* that later spread to developing fruit (Bahar et al., 2009, Latin and Hopkins, 1995, Hopkins and Thompson, 2002, Frankle et al., 1993). Watermelon fruit are most vulnerable to *A. citrulli* bacterium entering through stomata during the first five weeks post anthesis, prior to waxy cuticle stomatal plugging blocking *A* (Frankle et al., 1993). Fruit symptoms quickly progress from inconspicuous water-soaked lesions with irregular margins to expanded dark-green lesions that fissure, causing massive internal infection by secondary organisms, which leads fruit collapse (Latin and Hopkins, 1995, Hopkins et al., 1993). Seeds are internally and externally infested with *A. citrulli* bacteria (Rane and Latin, 1992), completing the disease cycle. Infestation can occur in symptomatic fruit or asymptomatic fruit exposed to the pathogen (Hopkins et al., 1996, Carvalho et al., 2013) or through a blossom infestation pathway (Walcott et al., 2003) Seed contamination does not appear to be affected by storage under dry conditions over 12 months and would likely not be eliminated by longer-term storage (Hopkins et al., 1996). Indeed, we have observed infected seedlings from four-year-old seed stored under cool, dry conditions. Insidious seed infestation continues to spread the pathogen around the world, representing a continuing serious threat to the watermelon industry.

Despite many resistance studies in watermelon (Hopkins et al., 1993, Hopkins and Thompson, 2002, Carvalho et al., 2013, Ma and Wehner, 2015, Hopkins and Levi, 2008, Sowell

and Schaad, 1979), there are no resistant commercial lines available (Johnson et al., 2011) and BFB resistance inheritance and mechanisms are little understood. However, Hopkins and Levi (2008) described the partial introgression of seedling resistance from two PIs (Hopkins and Thompson, 2002) into Crimson Sweet; they concluded that resistance was complexly inherited but was attainable through progeny testing during backcrossing and inbred production Such foliar resistance is likely predominantly determined by intricate avirulence-resistance gene interactions that are greatly affected by host and pathogen genetics. In contrast to foliar resistance, fruit resistance may be based primary on an entirely different set of genes affecting fruit cuticle accumulation that leads to stomatal plugging which blocks *A. citrulli* ingress (Frankle et al., 1993). Fruit resistance would then be a barrier resistance that would be irrespective of evolving *A. citrulli* pathogenicity genes and watermelon resistance genes. As far as investigated, genetic control and inheritance of cuticle formation in watermelon is unknown. An understanding of watermelon fruit resistance heritability and testing variance components would facilitate future breeding and resistance mechanism research.

In this study, we estimated broad-sense heritability (H^2) and variance components for fruit resistance among *Citrullus* spp. plant introductions tested under field conditions. Using these estimates, we calculated broad-sense heritabilities and error variances for hypothetical scenarios in order to identify an optimized experimental design for BFB fruit resistance breeding.

2 MATERIALS AND METHODS

2.1 Broad-Sense Heritability Estimates

Broad-sense heritability estimates and variance components were based on *Citrullus* spp. fruit resistance screening data collected at the Horticultural Crops Research Station in Clinton,

NC and described in Chapter 2. Broad-sense heritability was calculated using the general equation:

Equation 4.1: $H^2 = \frac{\sigma_G^2}{[\sigma_G^2 + \sigma_E^2]}$

Broad-sense heritability estimates and standard errors of five subsets of the data based on observation cutoff values, two, three, four, five, and six, were calculated using the 'nadiv' 2.14.3.1 (Wolak, 2012) pin() function on variance components from the ASREML-R model in which all components were designated as random effects: Cultigen, Year x Cultigen, Block(Year), Block(Year) x Cultigen, and Date[Block(Year)]. In some subsets, the Year x Cultigen random effect was not significant (p = 0.05) according to REML likelihood ratio tests provided by the 'asremlPlus' 2.0-12 (Brien, 2016) function *reml.lrt.asreml()* and was removed from the analysis. In order to account for unbalanced data, we used harmonic means (HM) to approximate years, blocks within years, and replications (Holland et al., 2003) (Table 4.1):

Equation 4.2: $H^2_{Calculated} =$

$$\frac{\sigma^2_{Cultigen}}{\sigma^2_{Cultigen} + \frac{\sigma^2_{Cultigen \times Year}}{HM_{Years\ per\ Cultigen}} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{HM_{Block(Year)\ per\ Cultigen}} + \frac{\sigma^2_{Error}}{HM_{replications\ per\ cultigen}}}$$

The data for each variance component and heritability estimate across averaged across each subset to generate a general estimate for optimization analysis.

2.2 Experiment Optimization

The averages of each variance component across the five data subsets were used to calculate estimates of σ^2_{Error}, H^2, and H^2 per year over 15 allocation scenarios. In each scenario different allocations of lines, blocks within year, and replications with no missing values were used to calculate simulated broad-sense heritability estimates:

Equation 4.3: $\sigma^2_{Simulated\ Error} = \frac{\sigma^2_{Block(Year)}}{Blocks\ per\ Year} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{Blocks\ per\ Year} + \frac{\sigma^2_{Error}}{Years \times Blocks \times Replications}$

Equation 4.4: $H^2_{Simulated} = \frac{\sigma^2_{Cultigen}}{\sigma^2_{Cultigen} + \frac{\sigma^2_{Cultigen \times Year}}{Year} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{Blocks} + \frac{\sigma^2_{Error}}{Years \times Blocks \times Replications}}$

3 RESULTS

3.1 Broad-sense Heritability and Variance Component Estimates

Harmonic means for years, blocks within year, and replications across all years for each cultigen increased as the number of minimum observations in each subset increased (Table 4.1). The overall increase in harmonic means contributed to slightly higher $H^2_{Calculated}$ estimates which ranged from 0.241 to 0.313 with an average of 0.276 (Table 4.2). The $\sigma^2_{Cultigen}$ and σ^2_{Error} components decreased from 0.509 to 0.358 and 3.476 to 3.088, respectively, as the minimum observations increased. As for the other variance components, as the minimum observations increased, $\sigma^2_{Cultigen \times Year}$ and $\sigma^2_{Date[Block(Year)]}$ were static at an average of 0.172 and 0.252, respectively; $\sigma^2_{Block(Year)}$ trended upward, 0.367 to 0.422; and $\sigma^2_{Block(Year) \times cultigen}$ trended downward, 0.828 to 0.671.

3.2 Broad-sense Heritability Experimental Optimization

Scenario 12 had the highest $H^2_{Simulated}$ and least $\sigma^2_{Simulated\ Error}$, and scenario 11 had the highest $H^2_{Simulated}$ per year, i.e., efficiency (Table 4.3). The $H^2_{Simulated}$ and $\sigma^2_{Simulated\ Error}$ for the five scenarios within each set of lines did not change linearly as years were doubled and the number of blocks within a year decreased by a half; instead, both $H^2_{Simulated}$ and $\sigma^2_{Simulated\ Error}$ changed as inverse curves to each other with their respective maximum and minimum values occurring within each set of lines at two years and eight blocks within each year. The inverse relationship between $H^2_{Simulated}$ and $\sigma^2_{Simulated\ Error}$ was further confirmed by the strong negative correlation (Pearson's product-moment correlation, $r(13) = -0.92$, $p < .0001$). $H^2_{Simulated}$ per year did decrease nearly linearly within each set of lines. The worst $H^2_{Simulated}$,

$\sigma^2_{Simulated\ Error}$, and $H^2_{Simulated}$ per year within each set of lines was when year was maximized at 16. As replications increased there was an overall increase in $H^2_{Simulated}$ and $H^2_{Simulated}$ per year and a decrease in $\sigma^2_{Simulated\ Error}$ between line sets. When adjusted from 1 to 2 replications the average $H^2_{Simulated}$ increased from 0.46 to 0.51 and from 2 to 4 replications, $H^2_{Simulated}$ increased to 0.54, with overall gain of 18.6% in heritability when adjusted from 1 to 4 replications. The gain in $H^2_{Simulated}$ was even more pronounced when considering only the maximum $H^2_{Simulated}$ within each line set, scenarios 2,7, and 12, in which the increase when going from 1 to 4 replications increased $H^2_{Simulated}$ from 0.54 to 0.66, a 22.2% gain.

4 DISCUSSION

4.1 Broad-sense Heritability and Experimental Optimization

The $H^2_{Calculated}$ estimates were strongly influenced by the number of replications, years, and blocks within each year. Overall, the general increase of $H^2_{Calculated}$ as the number of low-replication lines decreased had two causes: 1-high-replication lines had higher harmonic means for years, blocks within year, and replications than low-replication lines (Table 4.1); 2- higher-replication lines had generally decreased σ^2_{Error} and $\sigma^2_{Block(Year) \times cultigen}$ estimates (Table 4.2). Interestingly, there was a corresponding decrease in the $\sigma^2_{Cultigen}$ which was because the narrower higher-replication subsets have fewer extreme susceptible ratings (data not shown). For example, the predicted means and standard deviations for the low-replication (2 > obs) and high replication (6 > obs) subsets were 4.6 and 1.5, and 3.8 and 0.7, respectively. The decrease in $\sigma^2_{Cultigen}$ in the high-replication groups did negatively affect H^2 but did not offset the gains from the decrease in other variance components and increase in harmonic means.

In this study, we focused on H^2 as the metric for an optimized breeding program because of its importance in the breeder's equation. In addition, a decrease in σ^2_{Error} increases the

statistical power to differentiate between lines, which is extremely important as breeders seek to demonstrate line superiority over prior germplasm. Because error variance components (Equation 4.3) are part of the denominator in the H^2 calculations (Equation 4.4), we were unsurprised by the strong negative correlation in our scenarios. Consequently, breeding programs that seek to maximize heritability should target major sources of variation.

We simulated alternative resource allocations and calculated $H^2_{Simulated}$ in order to identify optimized testing conditions. The best scenario would take the shortest amount of time (fewest years) and generate sufficiently high H^2 and low σ^2_{Error}. Since year was a minor source of variation and blocks within year was a major source of variation, increasing years while decreasing blocks was detrimental to $H^2_{Simulated}$ beyond two years. Indeed, scenarios that maximized years had the lowest $H^2_{Simulated}$ and highest $\sigma^2_{Simulated\ Error}$ estimates. Although the maximum heritability for each set of lines was two years with eight testing blocks per year within each set of lines, running a large study of 16 blocks during a single year yielded the maximum $H^2_{Simulated}$ per year and thus the highest expected genetic gain per year. The number of replications did impact the $H^2_{Simulated}$ significantly, but the gain was modest when adjusting from two to four replications. Another breeding consideration is that the 22% gain in $H^2_{Simulated}$ going from one replication of 1000 lines to four replications of 250 lines will negatively affect population diversity under high selection intensity, e.g., 10% selection intensity will leave 100 and 25 lines, respectively. Being able to use a stringent selection intensity on the widest possible set of lines could mitigate the genetic gain loss from the slightly lower heritability associated with lower replications. Optimally, testing at 1 year with 16 blocks and 2 replications minimizes time, doesn't severely narrow the number of lines tested, and provides a high H^2 per year. Ultimately, the scenarios described in Table 4.3 provide only a sample of all of the possible

allocations that can be made and should be a general guide for breeders as they calculate the ideal design based on their resources.

4.2 Broad-sense Heritability and Genetic Control

Although we have evidence of a polygenic inheritance for seedling resistance (Hopkins and Levi, 2008), care must be taken when extrapolating the same genetic control to another growth stage or organ, i.e., the fruit. For example, in two inheritance studies on resistance to powdery mildew *Podosphaera xanthii* race 2W, stem and leaf resistance were shown to have alternative genetic control and differing narrow-sense heritability estimates (Tetteh et al., 2013a, Tetteh et al., 2013b). In the case of BFB, foliar resistance appears to be dependent on the developmental stage (Carvalho et al., 2013). Moreover, fruit resistance is further complicated by the possibility that the probable mechanism for fruit resistance is cuticle based rather than resistance gene mechanisms. Intriguingly, because this resistance could be based on stomatal plugging (Frankle et al., 1993), inheritance would be for genes that affect cuticle accumulation. As far as investigated, there is no literature concerning cuticle genetic control in watermelon or inheritance. However, cuticle regulation in general has been heavily investigated in other crops, and the research suggests intricate regulatory genes that are strongly affected by environmental factors (Yeats and Rose, 2013), which would partially explain our low H^2 estimates if cuticle is the fruit resistance mechanism. It's possible that homologous genes affect watermelon cuticle formation and that these genes provide a starting point in understanding genetic control of cuticle formation in watermelon and its inheritance.

4.3 Improving Heritability Estimates

Our resistance breeding study did not involve making crosses so we were only able to estimate total genetic variance, $\sigma^2_{Cultigen}$, that includes additive, dominance, and epistatic

variances. Ultimately, breeding designs for BFB fruit resistance depend on narrow-sense heritability estimates (h^2) which are based on additive variance. Future studies could partition the genetic variance into additive and dominance source using designs such as North Carolina Design I, North Carolina Design II (Comstock and Robinson, 1948), and North Carolina Design III (Comstock and Robinson, 1952). Because BFB resistance h^2 is a subset of our already low $H^2_{Calculated}$ estimate, gains will require high replication testing on advanced generations using an optimized breeding design.

Alternatively, if the fruit resistance can be definitively correlated with cuticle formation, resistant varieties could be indirectly selected for by identifying lines with early stomatal plugging. Such studies could be conducted in greenhouses where fruit ages could be exactly recorded and environmental effects would be minimized compared to the field resistance method. The additional control and precision would lead to higher heritability estimates, increased gain over time, and smaller study sizes.

5 CONCLUSION

In this study, we provided H^2 estimates based on a field BFB fruit resistance screening and explored ways to enhance heritability by adjusting experiment allocations. Our low average $H^2_{Calculated}$, of which h^2 is an unknown portion, suggests that the resistance is very environmentally-dependent and would require high replication testing over many locations and in advanced generations. Our H^2 and variance estimates are unique to our particular testing conditions but are applicable to future studies under similar conditions. In our allocation scenarios, our $H^2_{Simulated}$ based on a one-year study that maximized blocks and was adjusted for four replications was more than double our $H^2_{Calculated}$. The same design with fewer replications would provide a wider screening with acceptably minor heritability loss that could be offset by a

higher selection intensity. Alternative screening methods for BFB fruit resistance, such as those targeting early stomatal plugging, would increase heritability estimates by increasing the test objectivity and precision and reducing environmental effects.

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Table 4.1. Harmonic means of years, blocks within year, and replications across all years for each cultigen.

Minimum obs[z]	Years	Blocks(Year)	Replications
2	2.24	1.40	3.61
3	2.43	1.46	4.26
4	2.60	1.56	5.01
5	2.74	1.72	6.06
6	2.81	1.91	7.26
Average	2.56	1.61	5.24

[z]Minimum number of observations per line in each subset

Table 4.2. Broad-sense heritability and variance estimates for observation for data subsets based on minimum number of observations per line

Min. obs.	Number of Lines	H^{2z} Estimate	SE	$\sigma^2_{Cultigen}$ Estimate	SE	$\sigma^2_{Cultigen \times Year}$ Estimate	SE	$\sigma^2_{Block(Year)}$ Estimate	SE	$\sigma^2_{Block(Year) \times cultige}$ Estimate	SE	$\sigma^2_{Date[Block(Year)]}$ Estimate	SE	σ^2_{Error} Estimate	SE
2	1357	0.241	0.029	0.509	0.071	0.121	0.103	0.367	0.178	0.828	0.199	0.253	0.060	3.476	0.179
3	1143	0.266	0.030	0.518	0.065	NS[y]	-	0.351	0.172	0.898	0.185	0.257	0.062	3.462	0.177
4	841	0.265	0.034	0.438	0.064	NS	-	0.368	0.179	0.845	0.178	0.253	0.063	3.369	0.171
5	500	0.313	0.042	0.447	0.073	NS	-	0.398	0.194	0.779	0.172	0.257	0.068	3.202	0.164
6	281	0.295	0.061	0.358	0.092	0.224	0.128	0.422	0.206	0.671	0.192	0.240	0.074	3.088	0.165
	Average	0.276	0.039	0.454	0.073	0.172	0.116	0.381	0.186	0.804	0.185	0.252	0.065	3.320	0.171

[z]Broad-sense heritability = $\dfrac{\sigma^2_{Cultigen}}{[\sigma^2_{Cultigen} + \frac{\sigma^2_{Cultigen \times Year}}{HM_{Years\ per\ Cultigen}} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{HM_{Block(Year)\ per\ Cultigen}} + \frac{\sigma^2_{Error}}{HM_{replications\ per\ cultigen}}}$

[y]Not significant at the p = 0.05 value using the REML Likelihood Ratio Test.

Table 4.3. Broad-sense heritability optimization scenarios using different allocations of years, blocks, and replications.

	Allocations				Estimate[z]		
Scenario	No. Lines	Years	Blocks[y]	Replications[x]	σ^2_{Error} [w]	H^2 [v]	H^2 per year
1	1000	1	16	1	0.40	0.51	0.51
2	1000	2	8	1	0.34	0.54	0.27
3	1000	4	4	1	0.35	0.50	0.13
4	1000	8	2	1	0.42	0.42	0.05
5	1000	16	1	1	0.60	0.31	0.02
6	500	1	16	2	0.30	0.58	0.58
7	500	2	8	2	0.24	0.61	0.31
8	500	4	4	2	0.24	0.57	0.14
9	500	8	2	2	0.32	0.46	0.06
10	500	16	1	2	0.50	0.33	0.02
11	250	1	16	4	0.25	0.62	0.62
12	250	2	8	4	0.19	0.66	0.33
13	250	4	4	4	0.19	0.61	0.15
14	250	8	2	4	0.26	0.49	0.06
15	250	16	1	4	0.44	0.34	0.02

σ^2_{Error} and H^2 correlation: -0.92[u]

[z]Variance estimates are the averages across five data subsets for each variance component (table 2.2): $\sigma^2_{Cultigen} = 0.454$; $\sigma^2_{Cultigen \times Year} = 0.172$; $\sigma^2_{Block(Year)} = 0.381$; $\sigma^2_{Block(Year) \times cultigen} = 0.804$; $\sigma^2_{Date[Block(Year)]} = 0.252$; $\sigma^2_{Error} = 3.320$

[y]Blocks per year

[x]Replications per block

[w]Error variance = $\frac{\sigma^2_{Block(Year)}}{Blocks\ per\ Year} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{Blocks\ per\ Year} + \frac{\sigma^2_{Error}}{Years \times Blocks \times Replications}$

[v]Broad-sense heritability = $\frac{\sigma^2_{Cultigen}}{\sigma^2_{Cultigen} + \frac{\sigma^2_{Cultigen \times Year}}{Year} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{Blocks} + \frac{\sigma^2_{Error}}{Years \times Blocks \times Replications}}$

[u]Pearson's product-moment correlation (p < .0001)

APPENDICES

Appendix A Survey of *A. citrulli* inoculations methods.

Study	Species[w]	Plant Dev Stage[x]	Inoculum Concentration (CFU/ml)	Inoculation Method	Growing Conditions[y]	# of Lines Tested	Research Objective
(GOTH and WEBB, 1981)	Watermelon	2-week-old seedlings	10^6	Foliar cotton rub	Greenhouse	38 Cultivars	Resistance screen
(SOWELL and SCHAAD, 1979)	Watermelon	2-week-old seedlings	10^6 (PIs) 10^7 (Cultivars)	Foliar spray	Greenhouse (PIs) Field (Cultivars)	540 PIs 16 Cultivars	Resistance screen
(SOWELL and SCHAAD, 1979)	Watermelon	Mature Fruit	10^8	Injection	Greenhouse	30 PIs	Pathogenicity screen
(RANE and LATIN, 1992)	Watermelon	10-12-day-old seedlings	10^7	Foliar spray, leaf infiltration, or needle stab	Greenhouse	1 Cultivar	Pathogenicity screen
(RANE and LATIN, 1992)	Watermelon	Fruit 2-5 weeks post pollination	10^7	Gauze spread across surface		1 Cultivar	Pathogenicity screen
(HOPKINS et al., 1993)	Watermelon	2-week-old seedlings	$10^{5\text{-}6}$	Cotyledon Rubbing or Spray	Greenhouse	22 Cultivars 2 PIs	Resistance screen
(FRANKLE et al., 1993)	Watermelon	Immature Fruit 1-2 weeks postanthesis	10^6	Fruit spray	Greenhouse	1 Cultivar	Fruit resistance mechanisms
(HOPKINS et al., 1993)	Watermelon	~7-week-old plants	$10^{5\text{-}6}$	Foliar spray	Field	18 Cultivars	Resistance screen
(HOPKINS et al., 1993)	Watermelon	Immature Fruit 10-20 & 35-35 days postanthesis	$10^{5\text{-}6}$	Fruit spray	Field	18 Cultivars	Disease incidence
(HOPKINS et al., 1996)	Watermelon	14-21 days prior to fruit maturation	10^6	Fruit spray	Field	1 Cultivar	Seed treatment screen and disease incidence
(HOPKINS et al., 2003)	Watermelon and melon	Seeds	$10^{6\text{-}7}$	Seed exposure	Laboratory/Field	2 Watermelon Cultivars 1 Melon Cultivar	Seed treatment screen and disease incidence
(HOPKINS and THOMPSON, 2002a)	##Various cucurbits	~1 week after anthesis	$10^{5\text{-}6}$	Fruit spray	Field	13 Various cultivars	Fruit symptom evaluation and seed transmission assay
(HOPKINS and THOMPSON, 2002b)	Watermelon and *Praecitrulllus fistulosus*	2-week-old seedlings	$10^{5\text{-}6}$	Foliar spray	Greenhouse	1,334 PIs	Resistance screen
(HOPKINS and THOMPSON, 2002b)	Watermelon and *Praecitrulllus fistulosus*	6-week-old Plants	$10^{5\text{-}6}$	Foliar spray	Field	80 PIs	Resistance screen
(WALCOTT et al., 2004)	Watermelon, melon, pumpkin, and squash	2-week-old seedlings	0.5×10^6	Foliar spray	Greenhouse	1 Cultivar of each species	Pathogenicity assay

Appendix A. Continued.

(Walcott et al., 2004)	Watermelon and melon	Immature Fruit 3-9 days after pollination	0.5 x 10^{6}	Cotton swab	Greenhouse	1 Cultivar of each species	Pathogenicity assay
(Burdman et al., 2005)	Melon and cucumber	Seeds	10^{8}	Seed exposure	Greenhouse	1 Cultivar of each species	Seed transmission assay
(Burdman et al., 2005)	Watermelon and melon	Harvested fruit	10^{6-8}	Fruit injection	Laboratory	1 Cultivar of each species	Isolate pathogenicity assay
(Hopkins and Levi, 2008)	Watermelon	6-10-day-old plants	10^{6}	Foliar spray	Greenhouse	BC lines	Resistance screen
(Bahar et al., 2009)	Melon	Seeds	10^{6}	Seed exposure	Greenhouse	15 Cultivars 20 PIs	Seed transmission assay
(Bahar et al., 2009)	Melon	Seedlings	10^{6}	Foliar spray	Greenhouse	15 Cultivars 20 PIs	Resistance screen
(Bahar et al., 2009)	Melon	10-14 day old plants	10^{6}	Foliar spray	Field	4 Cultivars 4 PIs	Resistance screen
(Wechter et al., 2011)	Melon	Seeds	4-5 x 10^{8}	Vacuum- infusion	Laboratory	332 PIs 1 Cultivar	Resistance screen
(Wechter et al., 2011)	Melon	Second fully expanded leaf	10^{6}	Foliar spray	Growth Chamber	16 PIs 1 Cultivar	Resistance screen
(Dutta et al., 2012)	Watermelon	Ovaries at anthesis	10^{6}	Cotton swab	Greenhouse	1 Cultivar	Seed colonization study
(Carvalho et al., 2013)	Watermelon	Seed	3.4 x 10^{7}	Seed exposure	Greenhouse	63 PIs 11 Cultivars	Resistance screen
(Carvalho et al., 2013)	Watermelon	14-day-old plants	3.4 x 10^{7}	Foliar spray	Greenhouse	20 PIs 9 Cultivars	Resistance screen
(Carvalho et al., 2013)	Watermelon	5-week-old plants (prior to flowering)	3.4 x 10^{7}	Foliar spray	Greenhouse	20 PIs 9 Cultivars	Resistance screen
(Carvalho et al., 2013)	Watermelon	7-week-old plants (flowering and fruiting stage)	3.4 x 10^{7}	Foliar spray	Greenhouse	4 PIs 3 Cultivars	Resistance screen
(Carvalho et al., 2013)	Watermelon	Fruit of 8-week-old plants	3.4 x 10^{7}	Fruit spray	Greenhouse	4 PIs 3 Cultivars	Seed transmission assay Disease incidence
(Tian et al., 2015)	Melon	Seeds	10^{6}	Seed exposure	Laboratory	1 Cultivar	Seed colonization study (mutant)

Appendix A. Continued.

(DUTTA et al., 2015)	Watermelon	Ovaries at anthesis	10^6	Cotton swab	Greenhouse	1 Cultivar	Colonization pathway study
(DUTTA et al., 2015)	Watermelon	Stigma at anthesis	10^{7-8}	Pipette	Greenhouse	1 Cultivar	Colonization pathway study
(MA and WEHNER, 2015)	Watermelon	4-6 leaf stage	10^6	Natural spread and foliar spray	Field	1699 PIs 14 Cultivars	Resistance screening
(TIAN et al., 2016)	Watermelon	Seeds	$8 \times 10^{1-6}$	Seed exposure	Laboratory	1 Cultivar	Pathogen detection assay
(YAN et al., 2017)	Melon	6-day-old fruit	0.5×10^8	Pipette in to wounds	Laboratory	1 Cultivar	Isolate pathogenicity assay

[w] 'Watermelon', 'melon', 'squash', 'cucumber', and 'pumpkin' refer to *Citrullus spp., Cucumis spp., Cucurbita pepo, Cucumis sativus,* and *Cucurbita maxima.*

[x] The plant stage or target organ at time of inoculation

[y] Growing conditions at time of inoculation through evaluation

Appendix B. Bacterial fruit blotch disease severity and disease incidence for 1,357 Citrullus spp. cultigens with at least two observations.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 386024	colocynthis	Iran	-0.2	1.5	2	0-0	2	0	0	0
PI 386015	colocynthis	Iran	-0.1	1.5	2	0-0	2	0	0	0
PI 596665	amarus	South Africa, Transvaal	0.2	1.6	2	0-0.5	2	1	0	0
PI 482261	amarus	Zimbabwe	0.3	1.2	3	0-0.5	3	0	0	0
PI 482285	lanatus	Zimbabwe	0.4	1.5	2	0-1	2	0	0	0
PI 386025	colocynthis	Iran	0.4	1.3	3	0-1	3	0	0	0
PI 494819	lanatus	Zambia	0.4	0.7	22	0-1	22	1	0	1
PI 288316	amarus	India, Gujarat	0.4	1.5	2	0-0	2	0	0	0
Grif 16135	amarus		0.5	1	5	0-1	5	0	1	0
PI 273480	lanatus	Ethiopia	0.5	1.5	2	0-0	2	0	0	0
PI 164543	lanatus	India, Rajasthan	0.5	1.5	2	0-1	2	0	0	0
PI 177320	lanatus	Turkey, Ankara	0.5	1.5	2	1-1	2	0	0	0
PI 255137	amarus	South Africa, Transvaal	0.5	1.5	2	0-0.5	2	0	0	0
PI 271771	amarus	South Africa, Transvaal	0.6	1.3	3	0.3-2.5	2	1	1	1
PI 596677	amarus	South Africa	0.6	1.3	3	0.1-1.8	3	1	0	0
PI 357657	lanatus	Macedonia	0.6	1.5	2	0-2	2	1	1	1
PI 296342	amarus	South Africa, Cape Province	0.6	1.1	4	0-1	4	1	0	0
PI 526231	lanatus	Zimbabwe	0.7	1	5	0-1	5	0	0	1
PI 269365	colocynthis	Afghanistan, Kandahar	0.7	1.5	2	0-1	2	0	0	0
PI 500308	amarus	Zambia	0.7	1.5	2	1-1	2	0	1	0
PI 500313	lanatus	Zambia	0.7	1.1	4	0-1	4	1	0	1
PI 271770	amarus	South Africa, Transvaal	0.7	1.1	4	0-2	4	1	1	0
PI 320988	lanatus	Sierra Leone	0.7	1.5	2	0-0	2	0	0	0
PI 596666	amarus	South Africa, Transvaal	0.7	0.8	9	0-1	9	2	0	0
PI 482312	amarus	Zimbabwe	0.9	1.5	2	0-1	2	1	1	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 381737	lanatus	India	0.9	1.5	2	1-1	2	0	0	1
PI 596659	amarus	South Africa	0.9	0.7	14	0-2.8	13	4	1	1
PI 482360	lanatus	Zimbabwe	0.9	1.1	4	0-2.2	3	1	1	1
PI 596671	amarus	South Africa	0.9	1	5	0-2	5	1	1	1
PI 482288	lanatus	Zimbabwe	1	1.3	3	0-1.3	3	0	0	0
PI 500353	lanatus	Zambia	1	1.3	3	0.5-2	3	0	2	0
PI 482328	lanatus	Zimbabwe	1	1.3	3	0.5-2	3	0	1	0
PI 296337	amarus	South Africa, Cape Province	1	1.1	4	0-3	3	0	0	0
PI 596670	amarus	South Africa, Cape Province	1	0.7	18	0-2	18	1	1	1
PI 295842	amarus	South Africa	1	1.1	4	0-2	4	0	0	0
PI 482276	amarus	Zimbabwe	1	1	5	0.5-1.5	5	2	3	1
PI 482277	amarus	Zimbabwe	1	1.1	4	0-3	3	1	1	0
PI 482282	amarus	Zimbabwe	1	1.5	2	0.5-1	2	0	1	0
PI 490384	mucosospermus	Mali	1.1	0.8	14	0-1.5	14	0	1	0
PI 596656	amarus	South Africa	1.1	0.8	14	0-3.5	13	5	0	1
PI 526238	lanatus	Zimbabwe	1.1	1	5	0-2	5	0	0	0
PI 600790	lanatus	U.S., Michigan	1.1	1.1	4	0-1	4	1	1	0
PI 549162	lanatus	Chad	1.1	1.3	3	0-2	3	0	0	0
PI 490381	mucosospermus	Mali	1.1	1	5	0-3	4	0	0	0
PI 482347	lanatus	Zimbabwe	1.1	0.7	31	0-3.8	29	0	2	0
PI 482284	lanatus	Zimbabwe	1.1	1.3	3	1-1.7	3	1	0	0
PI 500345	lanatus	Zambia	1.1	1.5	2	1-1.5	2	1	1	0
PI 494532	mucosospermus	Nigeria, Oyo	1.1	0.9	6	0-4	5	1	1	1
PI 482322	amarus	Zimbabwe	1.1	1	5	0-2	5	1	0	0
PI 482298	amarus	Zimbabwe	1.2	1.1	4	1-3	3	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 386018	colocynthis	Iran	1.2	1.5	2	0-1	2	1	1	0
PI 278012	lanatus	Turkey, Gaziantep	1.2	1.1	4	0-4	3	0	0	0
PI 532666	amarus	Swaziland	1.2	0.9	7	0-2.2	6	4	2	2
PI 560000	mucosospermus	Nigeria, Oyo	1.2	1.2	3	0-3	2	0	0	0
PI 482260	lanatus	Zimbabwe	1.2	1.1	4	0.8-1	4	0	0	0
PI 169267	lanatus	Turkey, Edirne	1.2	1.1	4	1-3	3	1	1	1
PI 277982	lanatus	Turkey, Antalya	1.2	1.5	2	0-1	2	0	0	0
PI 482367	lanatus	Zimbabwe	1.2	0.8	8	0-2.7	6	4	2	2
PI 296335	amarus	South Africa, KwaZulu-	1.2	0.8	7	0.1-2.8	6	3	2	1
PI 500321	lanatus	Zambia	1.2	1.3	3	0-1	3	0	1	0
PI 505590	lanatus	Zambia	1.3	0.9	6	0-3.3	4	2	1	2
PI 512397	lanatus	Spain, Cordoba	1.3	1.3	3	0-1.5	3	0	0	0
PI 482326	amarus	Zimbabwe	1.3	1.1	4	0-5	3	0	1	0
PI 482296	lanatus	Zimbabwe	1.3	0.8	9	0-2	9	2	3	2
PI 593345	lanatus	China, Shanghai	1.3	1.1	4	0-2	4	1	0	0
PI 169269	lanatus	Turkey, Kirklareli	1.3	1.1	4	0-2	4	1	1	0
PI 532722	mucosospermus	Zaire, Bas-Zaire	1.3	0.9	6	0.5-1.8	6	1	2	0
PI 494820	lanatus	Zambia	1.3	1	5	0-3	4	1	3	1
PI 482349	lanatus	Zimbabwe	1.3	0.9	6	0-4	5	1	0	0
PI 532667	amarus	Swaziland	1.3	1.5	2	0.7-1.5	2	1	0	0
PI 525081	amarus	Egypt, Qena	1.3	1.6	2	0-1.3	2	0	0	0
PI 227202	lanatus	Japan, Shizuoka	1.3	1.5	2	1-1.5	2	0	1	0
PI 169244	lanatus	Turkey, Antalya	1.3	1.1	4	0-2	4	0	1	2
PI 532723	mucosospermus	Zaire, Bas-Zaire	1.3	0.6	24	0-4.2	22	1	0	0
PI 271986	lanatus	Somalia	1.3	1.5	2	1-1.5	2	1	1	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 596675	amarus	South Africa	1.3	0.9	6	0-3	5	1	2	0
PI 482287	lanatus	Zimbabwe	1.3	1.3	3	0-3	2	1	0	1
PI 542118	amarus	Botswana	1.3	1.5	2	0-1	2	0	0	0
PI 271774	lanatus	South Africa, Transvaal	1.3	0.8	7	0-1.3	7	2	2	1
PI 386016	colocynthis	Iran	1.4	1.5	2	0-0	2	0	0	0
PI 536462	lanatus	Maldives	1.4	1.3	3	1-2	3	0	1	1
PI 482333	amarus	Zimbabwe	1.4	1.2	3	1-2.5	2	1	1	0
PI 357674	lanatus	Macedonia	1.4	1.1	4	1-2	4	1	2	1
PI 549160	lanatus	Chad	1.4	0.7	17	0-6	16	2	2	1
PI 293765	lanatus	Soviet Union, Former	1.4	1	5	0-3	4	0	1	0
PI 500319	lanatus	Zambia	1.4	0.7	17	0-9	13	2	1	2
PI 482264	lanatus	Zimbabwe	1.4	1.1	4	1-2	4	0	0	0
PI 180276	lanatus	India, Rajasthan	1.4	1.3	3	0.7-2	3	0	2	1
PI 600962	lanatus	NA	1.4	1.5	2	1-1	2	0	0	0
PI 381701	lanatus	India	1.4	0.9	7	0-2.5	6	0	1	1
PI 244019	amarus	South Africa, Transvaal	1.4	1.1	4	0-3.2	2	3	1	2
PI 500349	lanatus	Zambia	1.4	0.7	32	0-7	28	0	0	3
PI 512339	lanatus	Spain, Huesca	1.4	1.1	4	0-1	4	0	0	0
PI 542617	lanatus	Algeria	1.4	1.3	3	1-2	3	1	1	0
PI 596653	amarus	South Africa	1.4	1	5	0-2	5	3	2	1
PI 296334	amarus	South Africa, Limpopo	1.4	1.3	3	0-3	1	0	0	0
PI 482375	lanatus	Zimbabwe	1.5	0.9	6	1-2	6	1	1	1
PI 500331	amarus	Zambia	1.5	1.3	3	1-4	2	0	0	0
PI 482330	lanatus	Zimbabwe	1.5	1.2	4	1-4	3	0	1	0
PI 500346	lanatus	Zambia	1.5	1	5	0-4	4	0	0	0
PI 244018	amarus	South Africa, Transvaal	1.5	1	5	0.3-3	4	3	1	3

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 512371	lanatus	Spain, Alicante	1.5	1.2	3	1-2	3	1	1	2
PI 500312	lanatus	Zambia	1.5	0.6	36	0-6	29	2	0	1
PI 254735	mucosospermus	Senegal	1.5	0.9	7	0-4.7	6	1	2	0
PI 482252	amarus	Zimbabwe	1.5	1	5	0-4	4	2	2	1
PI 512373	lanatus	Spain, Alicante	1.5	1.1	4	0-4	3	0	0	0
PI 512398	lanatus	Spain, Granada	1.5	1.3	3	0-3	2	1	0	1
PI 271779	amarus	South Africa, Transvaal	1.5	1	5	0-4.5	4	0	1	0
PI 542123	amarus	Botswana	1.5	1.2	3	1-4	2	2	1	0
PI 482345	lanatus	Zimbabwe	1.5	1.3	3	0-3.2	2	0	1	1
PI 490380	mucosospermus	Mali	1.5	0.8	10	0-3	8	1	0	0
PI 296341	amarus	South Africa, Cape Province	1.5	1.3	3	1.2-2	3	2	2	0
PI 500310	lanatus	Zambia	1.5	1	5	0-2.5	4	0	0	0
PI 357710	lanatus	Macedonia	1.5	1.1	4	0-3	3	2	1	0
PI 532624	amarus	Zimbabwe	1.6	1.1	4	0-3.2	3	3	2	1
PI 505586	lanatus	Zambia	1.6	1.3	3	1-2.2	2	0	2	0
PI 193490	lanatus	Ethiopia	1.6	1.2	3	1-1	3	1	1	0
PI 482311	amarus	Zimbabwe	1.6	1	5	0-3	3	2	2	0
PI 295850	amarus	South Africa	1.6	1.5	2	1-2	2	1	1	0
PI 344066	lanatus	Turkey, Gaziantep	1.6	1.3	3	2-2	3	0	0	0
PI 247399	lanatus	Greece, Peloponnese	1.6	1	5	0-4.5	4	0	0	0
PI 500338	lanatus	Zambia	1.6	1.1	4	0-4.5	3	1	1	1
PI 512375	lanatus	Spain, Alicante	1.6	1.2	3	0-2.5	2	1	1	1
PI 542115	lanatus	Botswana	1.6	0.9	6	0-3	4	4	2	1
PI 512354	lanatus	Spain, Toledo	1.6	1	5	0-2.7	4	1	1	1
PI 357703	lanatus	Macedonia	1.6	1.1	4	0-1	4	0	0	0
PI 534586	lanatus	Syria	1.6	0.9	7	0-3.4	4	1	2	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 494821	lanatus	Zambia	1.6	1	5	0-2	5	1	1	1
PI 549163	lanatus	Chad	1.6	1	5	1-2	5	2	2	1
PI 490386	mucosospermus	Mali	1.6	1	5	0-3	4	0	0	0
PI 278027	lanatus	Turkey, Kirklareli	1.6	1.1	4	0-4	3	1	1	0
Grif 15897	amarus	Russian Federation	1.6	1.3	3	0.5-4	2	1	0	0
PI 357683	lanatus	Macedonia	1.6	1.3	3	1-3	2	0	0	0
PI 596668	amarus	South Africa, Transvaal	1.6	0.7	14	0-6	10	1	0	1
PI 357747	lanatus	Macedonia	1.6	1.2	3	1-2	3	1	1	0
PI 525083	amarus	Egypt	1.6	1.1	4	0-5	3	1	1	0
PI 379256	lanatus	Macedonia	1.7	1.5	2	1-1	2	0	1	0
PI 549161	colocynthis	Chad	1.7	1.5	2	0-4	1	0	0	0
PI 179662	lanatus	India, Maharashtra	1.7	1.5	2	1-2.5	1	1	1	1
PI 185636	lanatus	Ghana	1.7	0.9	6	0-3	3	2	1	2
PI 169290	lanatus	Turkey, Bursa	1.7	1.3	3	1-2	3	1	1	1
PI 595203	mucosospermus	U.S., Georgia	1.7	1	5	0-3	3	0	1	0
PI 271773	amarus	South Africa, Transvaal	1.7	1.1	4	0-3	2	2	1	0
PI 278018	lanatus	Turkey, Izmir	1.7	1	5	0-3	4	1	1	1
PI 177327	lanatus	Turkey, Hakkari	1.7	1.1	4	0-4	2	0	0	0
PI 505604	amarus	Zambia	1.7	0.9	6	0-4	4	0	1	0
PI 254738	lanatus	Senegal	1.7	0.9	6	0-2.5	5	0	1	0
PI 482373	lanatus	Zimbabwe	1.7	0.8	7	1-3	5	2	2	0
PI 482365	lanatus	Zimbabwe	1.7	0.6	35	0-6	30	2	5	0
PI 276657	lanatus	Russian Federation	1.7	1.2	3	0-1	3	1	1	0
PI 490377	mucosospermus	Mali	1.7	0.8	9	0-8	8	1	0	1
PI 560015	mucosospermus	Nigeria, Oyo	1.7	0.8	15	0-6	13	1	1	0
PI 596669	amarus	South Africa, Cape Province	1.7	1	5	0-4.5	4	1	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 612461	lanatus	Korea, South, Pusan	1.7	1.3	3	1-3.3	2	0	1	1
PI 357717	lanatus	Macedonia	1.8	1.1	4	0-5	3	1	0	0
PI 658554	lanatus	Turkmenistan	1.8	0.7	14	0-7	11	1	1	2
PI 500336	lanatus	Zambia	1.8	0.9	6	1-2.5	5	1	2	1
PI 532664	amarus	Swaziland	1.8	0.9	6	0-3	3	4	1	3
PI 212209	lanatus	Greece	1.8	1	5	0-4	4	0	1	1
PI 505585	lanatus	Zambia	1.8	1	5	0.5-4.5	4	2	2	1
PI 368504	lanatus	Macedonia	1.8	1	5	0-3.5	4	1	1	0
PI 482350	lanatus	Zimbabwe	1.8	1.1	4	0-4	3	0	1	0
PI 596696	amarus	South Africa, Transvaal	1.8	1	5	0-4	3	2	1	0
PI 482344	lanatus	Zimbabwe	1.8	0.7	18	0-8	15	3	1	1
PI 189317	lanatus	Nigeria	1.8	0.8	7	0-3	5	0	1	1
PI 494527	mucosospermus	Nigeria, Ogun	1.8	0.8	9	0-3.5	6	2	0	0
PI 278037	lanatus	Turkey, Mardin	1.8	1.2	3	0-3	2	0	0	0
PI 306782	mucosospermus	Nigeria	1.8	0.9	7	0-4.8	4	2	1	2
PI 176907	lanatus	Turkey, Samsun	1.8	1.1	4	0.5-3	3	1	1	0
PI 270562	amarus	South Africa	1.8	0.9	6	0-4	3	2	2	0
PI 485579	amarus	Namibia	1.8	1.1	4	0-3	2	0	0	0
PI 482320	lanatus	Zimbabwe	1.8	1.1	4	1-3.5	2	1	2	1
PI 357663	lanatus	Macedonia	1.8	1	5	0-5	4	0	0	1
PI 542122	lanatus	Botswana	1.8	1.3	3	0-3.5	1	1	0	0
PI 532733	mucosospermus	Zaire, Bandundu	1.8	0.8	7	0-3	4	1	1	0
PI 518606	lanatus	Russian Federation	1.8	1.1	4	1-3	2	1	1	1
PI 357752	lanatus	Macedonia	1.8	1.5	2	0-3.5	1	0	0	0
PI 357699	lanatus	Macedonia	1.8	1.1	4	0-4	3	0	2	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 635699	lanatus	U.S., South Carolina	1.8	1.5	2	1-3	1	0	0	0
PI 635637	lanatus	U.S., Illinois	1.9	1.1	4	0-4	3	0	0	1
PI 277279	lanatus	India	1.9	1.1	5	0-7	3	1	1	1
PI 512360	lanatus	Spain, Caceres	1.9	1.1	4	0-3	3	0	0	1
PI 596676	amarus	South Africa	1.9	1	5	0-4	3	1	2	0
PI 500328	lanatus	Zambia	1.9	0.9	6	1-4	4	0	3	1
PI 296339	amarus	South Africa, Cape Province	1.9	0.8	8	0-3.7	5	1	2	0
PI 482293	amarus	Zimbabwe	1.9	1.1	4	1-5	3	1	1	1
PI 560005	mucosospermus	Nigeria, Oyo	1.9	1	5	1.5-3	4	1	1	0
PI 542114	amarus	Botswana	1.9	0.9	7	0-5	5	4	3	1
PI 512363	lanatus	Spain, Caceres	1.9	1	5	0-4.5	4	0	0	2
PI 253174	lanatus	Serbia	1.9	1	5	1-3	3	0	1	2
PI 534534	lanatus	Syria	1.9	1.2	3	1-4	2	1	1	0
PI 482272	lanatus	Zimbabwe	1.9	0.8	7	0-3	4	1	1	1
PI 595200	lanatus	U.S., Georgia	1.9	0.8	7	1-3.8	5	2	0	1
PI 357665	lanatus	Macedonia	1.9	1.5	2	0-2.8	1	0	0	0
PI 167125	lanatus	Turkey, Adana	1.9	1.3	3	0.5-3	2	0	1	0
PI 183218	lanatus	Egypt, Giza	1.9	0.9	6	1-4	3	2	3	1
PI 593373	lanatus	China, Xinjiang	1.9	1.1	4	0-5	3	1	1	0
PI 174101	lanatus	Turkey, Mardin	1.9	1.5	2	0.5-3	1	0	1	0
PI 271466	lanatus	India, Rajasthan	1.9	1.3	3	1-3.3	2	1	1	1
PI 228238	lanatus	Israel	2	1.3	3	1-4	2	0	0	1
PI 482294	lanatus	Zimbabwe	2	0.9	6	0-4	4	1	2	0
PI 179881	amarus	India, Gujarat	2	1.2	3	1-2	3	0	0	0
PI 169236	lanatus	Turkey, Manisa	2	1.5	2	2-3	1	1	0	0
PI 595202	lanatus	U.S., Georgia	2	0.8	8	0-5.2	5	1	0	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 278004	lanatus	Turkey, Bursa	2	1.1	4	1-3	2	1	1	1
PI 379255	lanatus	Macedonia	2	1.1	4	0-3	3	2	0	1
PI 288232	lanatus	Egypt	2	1.5	2	1-3	1	1	2	0
PI 182178	lanatus	Turkey, Tekirdag	2	0.9	11	0-6.5	7	2	1	1
PI 560013	mucosospermus	Nigeria, Ogun	2	0.9	6	1-3	4	1	1	0
PI 485580	amarus	Botswana	2	1.6	2	1-4	1	1	0	1
PI 660975	lanatus	Turkmenistan	2	0.8	11	0-8	5	1	4	2
PI 171392	lanatus	South Africa, Transvaal	2	0.9	6	0-3	4	3	0	0
PI 635670	lanatus	U.S., Wyoming	2	0.9	6	0-4	3	1	1	1
PI 536451	lanatus	Maldives	2	1.5	2	0-5	1	0	0	0
PI 420320	lanatus	Italy	2	1.3	3	0-3	2	1	0	0
PI 512393	lanatus	Spain, Castellon de Plana	2	1.3	3	1-3	2	1	2	1
PI 482346	lanatus	Zimbabwe	2	1	5	1-3	3	2	2	0
PI 254431	lanatus	Lebanon	2	0.9	6	0-4	4	1	0	1
PI 490376	lanatus	Mali	2	0.8	12	0-5	8	3	1	0
PI 176486	lanatus	Turkey, Tunceli	2	1.2	3	1-3	2	1	0	0
PI 364460	lanatus	South Africa, Limpopo	2	0.9	6	1-4	3	0	1	1
PI 169288	lanatus	Turkey, Bursa	2	1.1	5	1-3	3	0	1	1
PI 634691	lanatus		2	1.3	3	1-3	2	1	2	0
PI 494815	lanatus	Zambia	2	1.1	4	0-5	3	1	1	1
PI 532668	amarus	Swaziland	2	0.8	7	0-5	5	3	2	1
PI 560020	mucosospermus	Nigeria, Oyo	2	0.7	17	0-6.7	12	2	1	0
PI 172786	lanatus	Turkey	2	1.1	4	0-5	3	0	1	0
PI 254736	mucosospermus	Senegal	2	1	6	0-6	5	0	0	0
PI 593359	lanatus	China, Shaanxi	2	1.5	2	1-2	2	0	0	0
PI 278046	lanatus	Turkey, Nigde	2	1	5	0-2.5	4	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 171587	lanatus	Turkey, Artvin	2	1.1	4	1-2.8	3	1	0	0
PI 635630	lanatus	U.S., New Hampshire	2	1.1	4	0-3	3	1	1	1
PI 175663	lanatus	Turkey, Kayseri	2.1	1.3	3	0-4.5	2	0	0	1
PI 482275	lanatus	Zimbabwe	2.1	0.9	7	0.5-6	4	3	2	1
PI 635610	lanatus	U.S., California	2.1	1.5	2	0-3	1	0	0	0
PI 164685	lanatus	India, Tamil Nadu	2.1	1.5	2	0-3	1	0	0	0
PI 176910	lanatus	Turkey, Canakkale	2.1	1.1	4	1-2.5	3	0	0	0
PI 296343	amarus	South Africa, Cape Province	2.1	0.9	6	0-5	3	1	0	1
PI 482325	lanatus	Zimbabwe	2.1	1.3	3	0.9-4	2	0	0	0
PI 635698	lanatus	U.S., South Carolina	2.1	1.3	3	0-4	1	0	0	0
PI 612469	lanatus	Korea, South, Pusan	2.1	1.3	3	1-1.7	3	0	0	0
PI 635686	lanatus	U.S., Wyoming	2.1	1.5	2	2-3	1	1	1	0
PI 635640	lanatus	U.S., Florida	2.1	1.2	3	0-3	1	1	0	1
PI 534590	lanatus	Syria	2.1	1.2	3	0-4	2	0	0	0
PI 178874	lanatus	Turkey, Cankiri	2.1	0.8	7	0-6.5	5	1	1	0
PI 270525	lanatus	Israel	2.1	1.1	4	1-3	3	0	0	0
PI 525089	lanatus	Egypt, Sawhaj	2.1	1.2	3	0-2.5	2	0	0	0
PI 368506	lanatus	Macedonia	2.1	1.5	2	1-3	1	1	1	0
PI 537265	lanatus	Pakistan, Punjab	2.1	1	5	0-5	3	2	0	2
PI 560008	mucosospermus	Nigeria, Oyo	2.1	1.1	4	1.5-4	3	1	1	0
PI 438671	lanatus	Mexico, Yucatan	2.1	1.5	2	0-3	1	0	0	0
PI 482255	lanatus	Zimbabwe	2.1	1.3	3	1-3	2	0	0	1
PI 271769	amarus	South Africa, Transvaal	2.1	0.9	6	0-4.5	4	2	0	2
PI 174105	lanatus	Turkey, Gaziantep	2.1	1.5	2	2-3	1	1	1	0
PI 190050	lanatus	Serbia	2.1	1.6	2	1.5-2	2	0	0	0
PI 169282	lanatus	Turkey, Balikesir	2.2	1.5	2	0-4	1	1	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 278062	lanatus	Turkey, Zonguldak	2.2	0.9	7	1-4.5	5	0	1	1
PI 482305	lanatus	Zimbabwe	2.2	1	5	0-3.8	3	2	1	3
PI 505592	lanatus	Zambia	2.2	0.9	6	0-4	3	2	3	1
PI 379230	lanatus	Macedonia	2.2	1	5	0-4	3	1	1	0
PI 534533	lanatus	Syria	2.2	0.8	13	0-6	7	2	0	0
PI 635647	lanatus	U.S., Louisiana	2.2	1.3	3	1-5	2	1	0	1
PI 482359	lanatus	Zimbabwe	2.2	0.9	6	0-9	4	0	0	1
PI 482253	lanatus	Zimbabwe	2.2	1.1	4	1-2.7	1	1	0	1
PI 629106	lanatus	U.S., North Carolina	2.2	1.5	2	1-1.5	2	0	0	0
PI 500305	lanatus	Zambia	2.2	1.1	4	0-4.5	2	0	0	1
PI 277996	lanatus	Turkey, Bitlis	2.2	1.5	2	0-5	1	1	1	0
PI 278034	lanatus	Turkey, Maras	2.2	1	5	1-6	3	1	0	2
PI 494816	lanatus	Zambia	2.2	0.7	15	0-9	12	2	2	3
PI 379238	lanatus	Macedonia	2.2	1.3	3	2-3	2	1	1	0
PI 482292	lanatus	Zimbabwe	2.2	1.3	3	1-5	2	0	0	1
PI 179232	lanatus	Turkey, Tekirdag	2.2	1.3	3	1-2	3	0	0	1
PI 186489	mucosospermus	Nigeria	2.2	0.9	9	1-4	3	1	0	0
PI 635712	lanatus	U.S., Mississippi	2.2	1.5	2	2-3	1	0	0	1
PI 508443	lanatus	Korea, South	2.2	1.2	3	0-4.5	2	0	0	1
PI 357656	lanatus	Macedonia	2.2	0.8	12	0-7	6	1	1	2
PI 181868	lanatus	Syria	2.2	1.1	4	0-5	2	1	0	0
PI 176490	lanatus	Turkey, Sivas	2.2	1	5	1-4	3	1	2	1
PI 482247	lanatus	Zimbabwe	2.2	0.9	6	0-7	4	0	1	0
PI 172788	lanatus	Turkey, Trabzon	2.2	1	5	0-5	3	1	0	1
PI 482265	amarus	Zimbabwe	2.2	1.1	4	0-6	3	1	0	1
PI 276444	lanatus	Jordan	2.2	1.2	3	0-5	2	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 612473	lanatus	Korea, South, Pusan	2.2	1.1	4	1-2.5	3	1	1	2
PI 229604	lanatus	Iran, Mazandaran	2.2	1.3	3	1-3.3	2	1	2	1
PI 278047	lanatus	Turkey, Sakarya	2.2	1.1	4	0-3	2	0	0	0
PI 593364	lanatus	China, Xinjiang	2.2	0.9	8	1-5	6	0	2	1
PI 275628	lanatus	Pakistan, Northern Areas	2.2	1.3	3	0-5	1	0	0	1
PI 534583	lanatus	Syria	2.3	0.8	10	0-6	7	1	1	1
PI 606135	amarus	Russian Federation	2.3	1.1	4	0.5-5	2	0	1	1
PI 505587	lanatus	Zambia	2.3	0.6	39	0-8	29	5	2	8
Grif 15898	lanatus	U.S., Iowa	2.3	1	5	1-4	4	0	2	1
PI 482362	lanatus	Zimbabwe	2.3	1	5	0.3-7	4	3	3	1
PI 379243	amarus	Macedonia	2.3	1.1	5	1-3.8	1	0	1	0
PI 536446	lanatus	Maldives	2.3	1.3	3	1-3	2	0	1	1
PI 175653	lanatus	Turkey, Diyarbakir	2.3	1.2	3	1-3	2	1	1	1
PI 491265	lanatus	Zimbabwe	2.3	1.1	4	1-4.5	3	1	1	1
PI 277972	lanatus	Turkey, Adiyaman	2.3	0.9	6	0-3	4	3	0	1
PI 512399	lanatus	Spain, Granada	2.3	1.5	2	0-4	1	0	0	0
PI 635688	lanatus	U.S., Kansas	2.3	1.1	4	1-3	2	0	0	0
PI 500332	amarus	Zambia	2.3	1.5	2	0-4	1	0	0	0
PI 500317	lanatus	Zambia	2.3	1.1	4	0-6	3	1	1	0
PI 164708	lanatus	India, Karnataka	2.3	1.3	3	1-2.8	1	1	0	1
PI 254739	lanatus	Senegal	2.3	0.8	7	0.1-4.5	4	1	1	2
PI 534531	lanatus	Syria	2.3	1	5	0-4	3	0	1	0
PI 325248	lanatus	Soviet Union, Former	2.3	0.7	16	0-7	11	0	0	1
PI 560019	mucosospermus	Nigeria, Oyo	2.3	1	5	0-4	2	0	0	1
PI 564536	lanatus	U.S.	2.3	1.5	2	1-5	1	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 482295	lanatus	Zimbabwe	2.3	1	5	1-5	2	1	1	0
PI 500347	lanatus	Zambia	2.3	1.2	3	1-4	2	1	1	1
PI 482297	lanatus	Zimbabwe	2.3	0.9	6	0-6.5	4	2	3	2
PI 277971	lanatus	Turkey, Adiyaman	2.3	1.2	3	1-5.5	2	2	2	1
PI 482280	lanatus	Zimbabwe	2.3	1.1	4	0.5-4	1	3	2	1
PI 183673	lanatus	Turkey, Trabzon	2.3	1.1	4	0-3.5	2	1	1	0
PI 379246	lanatus	Macedonia	2.3	1.3	3	1-3	2	0	0	0
PI 186490	mucosospermus	Nigeria	2.3	0.9	6	1.5-3	2	2	2	0
PI 368513	lanatus	Montenegro	2.3	1	5	0-4	3	0	0	0
PI 482248	lanatus	Zimbabwe	2.3	0.8	7	0-5	4	1	2	0
PI 512332	lanatus	China, Beijing	2.3	0.9	6	1-5	5	1	2	1
PI 246029	lanatus	Chile, Bio-Bio	2.3	1.1	4	1-4	3	1	3	1
PI 275632	lanatus	India, Rajasthan	2.3	1.1	4	1-5	2	1	3	0
PI 169275	lanatus	Turkey, Canakkale	2.3	1.5	2	1-3	1	0	0	1
PI 505595	lanatus	Zambia	2.4	0.9	6	1-5	4	1	1	1
Allsweet	lanatus	NA	2.4	1.5	2	0-4	1	1	0	0
PI 536464	lanatus	Maldives	2.4	1.3	3	1-3	1	0	1	1
PI 307750	lanatus	Philippines, Luzon	2.4	0.7	15	0-6	7	1	0	4
PI 175660	lanatus	Turkey, Kirsehir	2.4	1.2	3	0.5-5	2	0	0	0
PI 357741	lanatus	Macedonia	2.4	1.3	3	1-5	2	1	1	0
PI 512854	amarus	Spain, Valencia	2.4	1.1	4	1-4.5	3	1	0	1
Grif 1730	lanatus	China, Jiangsu	2.4	1.2	3	0-3	2	1	1	1
PI 169241	lanatus	NA	2.4	1.5	2	2-3	1	0	0	0
PI 542120	lanatus	Botswana	2.4	1.5	2	1.5-4	1	1	0	1
PI 357750	lanatus	Macedonia	2.4	1.5	2	1-2	2	1	1	0
PI 295843	amarus	South Africa	2.4	1.2	4	0-5	1	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 357671	lanatus	Macedonia	2.4	1.2	3	1-3	2	0	0	2
PI 525097	lanatus	Egypt, Sinai	2.4	1	5	0-6	3	1	0	0
PI 169239	lanatus	Turkey, Manisa	2.4	1.1	4	0.5-6	3	1	1	0
PI 169296	lanatus	Turkey, Kocaeli	2.4	1.2	3	1-3	2	1	2	0
PI 195928	lanatus	Ethiopia	2.4	0.8	8	0-4	4	2	0	1
PI 169252	lanatus	Turkey, Aydin	2.4	1	5	0-5	4	0	1	1
PI 500311	lanatus	Zambia	2.4	1.1	4	1-4	2	0	0	1
PI 379224	lanatus	Macedonia	2.4	1.1	4	0-4	2	2	1	0
PI 635700	lanatus	U.S., South Carolina	2.4	1.3	3	2-4	1	0	0	0
PI 482250	lanatus	Zimbabwe	2.4	1.1	4	0.5-5	2	2	2	1
PI 500301	lanatus	Zambia	2.4	1	5	0-5.2	3	1	2	0
PI 299378	amarus	South Africa, Cape Province	2.4	1	5	0-5	2	1	1	0
PI 278051	lanatus	Turkey, Sivas	2.4	1.5	2	0-2.5	1	0	0	1
PI 560007	mucosospermus	Nigeria, Oyo	2.4	0.9	7	1-5	3	1	1	0
PI 277998	lanatus	Turkey, Bolu	2.4	1.5	2	1-3	1	0	1	0
PI 326515	lanatus	Ghana	2.4	1.3	3	0-5	2	0	0	0
PI 482348	lanatus	Zimbabwe	2.4	1	7	1-5.5	3	1	1	1
PI 368521	lanatus	Macedonia	2.4	1.1	4	0.5-5	2	0	0	0
PI189225	lanatus	NA	2.4	0.9	7	0-5	4	3	2	2
PI 485583	amarus	Botswana	2.4	1	5	0.7-3	2	3	3	0
Grif 1733	lanatus	China, Jiangsu	2.4	1.1	4	1-5.5	3	1	1	1
PI 379225	lanatus	Macedonia	2.4	1	5	0-4	2	1	2	0
PI 278049	lanatus	Turkey, Sinop	2.4	1	6	0-4	2	2	1	1
PI 381741	lanatus	India	2.4	1.5	2	0-2.5	1	0	1	0
PI 512395	lanatus	Spain, Valencia	2.4	1	6	0-9	3	2	0	1
PI 179233	lanatus	Turkey, Bursa	2.4	1	5	0-6	3	0	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 169294	lanatus	Turkey, Bursa	2.5	1.1	4	1-5	3	1	0	0
PI 525093	lanatus	Egypt, New Valley	2.5	1.1	4	0-3.5	3	2	2	1
PI 482257	amarus	Zimbabwe	2.5	0.9	6	0-5	3	2	3	1
PI 277992	lanatus	Turkey, Balikesir	2.5	1.5	2	2-3.5	1	1	1	0
PI 505594	lanatus	Zambia	2.5	1.1	4	1-5	2	0	2	1
PI 505593	lanatus	Zambia	2.5	1	5	0-4	2	1	1	0
PI 248178	mucosospermus	Zaire	2.5	0.8	7	1-4	3	2	2	0
PI 326516	mucosospermus	Ghana	2.5	0.8	9	1-4.5	5	3	4	0
PI 512389	lanatus	Spain, Valencia	2.5	1.3	3	1-3	2	0	1	1
PI 169300	lanatus	Turkey, Hatay	2.5	1.1	4	1-3	3	2	2	0
PI 537467	lanatus	Spain, La Palmas	2.5	0.8	9	0-5	5	0	1	1
PI 601662	lanatus	U.S., Florida	2.5	1.2	3	0-4	2	1	1	0
PI 357728	lanatus	Macedonia	2.5	1.3	3	1-3	1	0	0	1
PI 183023	lanatus	India, Maharashtra	2.5	1.3	3	0-5	2	1	1	1
Grif 5598	lanatus	India	2.5	1.3	3	2-3.5	1	0	0	1
PI 670011	rehmii		2.5	1	5	0-5	3	0	1	1
PI 536448	lanatus	Maldives	2.5	1.1	4	1-5	2	1	3	2
PI 176906	lanatus	Turkey, Urfa	2.5	1.1	4	1-3.8	1	1	2	1
PI 635590	lanatus	U.S., California	2.5	1.1	4	1-4	2	1	1	0
PI 357695	lanatus	Macedonia	2.5	1	5	0-5	3	2	1	1
Sugar Baby	lanatus	NA	2.5	0.8	13	0-6	10	0	1	2
PI 435991	lanatus	China, Shaanxi	2.5	1	5	1-3	4	1	1	2
PI 277985	lanatus	Turkey, Artvin	2.5	1.5	2	1.5-3	1	1	2	1
PI 482361	amarus	Zimbabwe	2.5	1.5	2	1-3.5	1	1	1	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 176914	lanatus	Turkey, Konya	2.5	0.8	7	0-4	4	2	3	2
PI 482249	lanatus	Zimbabwe	2.5	0.9	6	0-4	3	0	1	2
PI 370433	lanatus	Serbia	2.5	1.1	4	0.7-6	3	1	2	0
PI 381721	lanatus	India	2.5	0.9	6	0.5-5	3	1	3	0
PI 482310	lanatus	Zimbabwe	2.5	1.1	4	0-5	1	2	2	1
PI 227205	lanatus	Japan, Aichi	2.5	1	5	1-4	3	1	2	1
PI 500323	lanatus	Zambia	2.5	0.9	6	0-5.5	3	2	1	1
PI 635597	lanatus	U.S., California	2.5	1.3	3	1-2	3	0	1	0
PI 251515	lanatus	Iran, Esfahan	2.5	1.1	4	1-6	2	2	0	2
PI 532659	amarus	South Africa	2.5	0.9	6	0-8	3	2	2	3
PI 169278	lanatus	Turkey, Canakkale	2.5	1.3	3	0-5	2	2	1	1
PI 379237	lanatus	Macedonia	2.5	1.5	2	0-5.5	1	0	0	1
PI 430615	lanatus	China	2.5	1.1	4	0-3.5	2	0	1	0
PI 169264	lanatus	Turkey, Istanbul	2.5	1.1	4	0-5	2	0	0	1
PI 500354	amarus	Zambia	2.5	1.1	4	0-5.5	2	2	1	0
PI 512383	lanatus	Spain, Valencia	2.6	1	5	0-6	3	0	1	1
PI 169272	lanatus	Turkey, Edirne	2.6	1.3	3	1-4	2	0	1	0
PI 381695	lanatus	India	2.6	1	5	0-5	2	0	0	0
Grif 5599	lanatus	India	2.6	1	5	1-8	4	2	2	1
PI 271775	amarus	South Africa, Transvaal	2.6	1.3	3	0.5-7	2	0	2	1
PI 183399	lanatus	India, Madhya Pradesh	2.6	1.1	4	1-4	2	1	0	1
PI 175650	lanatus	Turkey, Balikesir	2.6	1.1	4	0-5	1	0	0	0
PI 181936	lanatus	Syria	2.6	1.1	4	1-5	2	1	2	1
PI 211915	lanatus	Iran, Tehran	2.6	1.1	4	1-5	2	1	2	0
PI 179879	lanatus	India, Rajasthan	2.6	1	5	0-5	3	1	2	1
PI 482278	lanatus	Zimbabwe	2.6	0.9	6	0-6	2	3	3	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 532818	lanatus	China	2.6	1.3	3	1-3	2	0	0	1
PI 482354	lanatus	Zimbabwe	2.6	1.1	4	0-5	2	0	0	1
PI 174107	lanatus	Turkey, Malatya	2.6	1.1	4	0-4	2	0	0	1
PI 500344	lanatus	Zambia	2.6	1.1	4	1-4	2	2	2	2
PI 482335	amarus	Zimbabwe	2.6	1	6	0-4	2	0	0	0
PI 177323	lanatus	Turkey, Amasya	2.6	1.2	3	1-3.8	2	2	2	1
PI 164248	mucosospermus	Liberia	2.6	0.8	7	0-5	3	2	2	1
PI 175654	lanatus	Turkey, Canakkale	2.6	1	5	1-5	4	1	1	1
PI 357729	lanatus	Macedonia	2.6	1.5	2	0-2.5	1	0	1	0
PI 176494	lanatus	Turkey, Konya	2.6	0.9	6	0-7	4	2	1	2
PI 278007	lanatus	Turkey, Gaziantep	2.6	1	5	0-6	4	1	1	1
PI 319235	lanatus	Japan	2.6	1.1	4	0-6	2	1	1	0
PI 504519	lanatus	Australia	2.6	1.1	4	0-7	3	1	1	1
PI 635620	lanatus	U.S., Mississippi	2.6	1.3	3	1-4	1	1	1	1
PI 525096	lanatus	Egypt	2.6	1.1	4	1-4.5	3	2	1	1
PI 277984	lanatus	Turkey, Antalya	2.6	1.5	2	1-5	1	1	0	1
PI 174100	lanatus	Turkey, Diyarbakir	2.6	1.5	2	2-3	1	0	0	2
PI 278008	lanatus	Turkey, Gaziantep	2.6	1	5	0-4.2	3	1	1	1
PI 357713	lanatus	Macedonia	2.6	1.3	3	1-6	2	0	0	1
PI 271747	lanatus	Afghanistan, Helmand	2.6	1.2	4	3-4	0	0	1	1
PI 512386	lanatus	Spain, Valencia	2.6	1	5	1-4	3	1	1	1
PI 459074	lanatus	Botswana	2.6	1	5	1-5	3	1	2	1
PI 512349	lanatus	Spain, Tarragona	2.6	1.1	4	0-4	2	0	0	0
PI 193963	lanatus	Ethiopia	2.6	1.3	3	1-4.7	1	1	2	0
PI 260733	lanatus	Sudan, Khartoum	2.6	0.9	6	0-4	4	2	1	1
PI 508445	lanatus	Korea, South	2.6	1.3	3	1-4	2	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 379231	lanatus	Macedonia	2.6	1.1	4	2-4	1	2	2	1
PI 182177	lanatus	Turkey, Kirklareli	2.6	1.1	4	1-4	2	1	1	1
PI 174108	lanatus	Turkey, Malatya	2.6	0.9	7	0-8	4	1	1	2
PI 635704	lanatus	U.S., Missouri	2.6	1.1	4	0-9	3	0	0	1
PI 249010	lanatus	Nigeria, Kaduna	2.6	1	5	1-4.5	4	0	1	1
PI 277997	lanatus	Turkey, Bingol	2.6	1.1	4	0-7	3	0	2	1
PI 560011	mucosospermus	Nigeria, Ogun	2.6	1	5	0-5	2	0	0	0
PI 537270	lanatus	Pakistan, Punjab	2.7	0.8	7	1-4.5	3	3	2	1
Grif 5596	lanatus	India	2.7	1.1	4	1-5	2	1	1	1
PI 593388	lanatus	China, Xinjiang	2.7	1.1	4	1-3.5	2	1	1	1
PI 512400	lanatus	Spain, Malaga	2.7	0.9	6	0-5	4	0	0	1
PI 500352	lanatus	Zambia	2.7	0.8	7	0-5	4	1	3	1
PI 500306	lanatus	Zambia	2.7	0.9	6	0-7	3	3	3	2
PI 482377	lanatus	Zimbabwe	2.7	0.9	6	1-4	2	1	1	2
PI 307748	lanatus	Philippines	2.7	1.2	3	0-4	2	0	0	0
PI 482352	lanatus	Zimbabwe	2.7	1.1	5	1-5.5	2	1	3	2
PI 169262	lanatus	Turkey, Manisa	2.7	1	5	2-4	2	1	1	2
Grif 14201	colocynthis	India, Rajasthan	2.7	1.3	3	0-7	2	1	0	1
PI 346787	lanatus	NA	2.7	1.5	2	1-3.5	1	0	0	1
PI 223765	lanatus	Afghanistan, Badakhshan	2.7	1.5	2	0-3	1	0	0	0
PI 537268	lanatus	Pakistan, Punjab	2.7	0.8	8	0-6	5	1	1	1
PI 172802	lanatus	Turkey, Urfa	2.7	1.2	3	0-4	1	1	1	1
PI 277970	lanatus	Turkey, Adiyaman	2.7	0.9	6	1-4	2	0	0	0
PI 293766	lanatus	Soviet Union, Former	2.7	0.9	11	0-8	5	1	2	3
Sangria	lanatus	NA	2.7	0.8	9	0-9	6	1	1	3
PI 482342	amarus	Zimbabwe	2.7	1.1	4	0-4	2	1	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 368516	lanatus	Macedonia	2.7	0.9	6	0-5	2	0	1	2
PI 370427	lanatus	Macedonia	2.7	1.1	4	0.3-5	1	0	1	1
PI 438676	lanatus	Mexico, Yucatan	2.7	1.1	4	1-7	2	0	0	0
PI 269464	lanatus	Pakistan, N.W. Frontier	2.7	1	5	1-5	2	1	1	0
PI 176909	lanatus	Turkey, Edirne	2.7	0.8	12	0-6	5	2	1	4
PI 368519	lanatus	Macedonia	2.7	1.1	4	1-4	1	0	2	0
PI 635613	lanatus	U.S., Colorado	2.7	1.1	4	1-5	2	1	1	0
PI 299379	amarus	South Africa, Cape Province	2.7	0.9	6	0-8	4	0	0	1
PI 357702	lanatus	Macedonia	2.7	0.8	7	0-6.5	4	1	3	1
PI 169255	lanatus	Turkey, Manisa	2.7	1	5	0-6.5	4	0	1	1
PI 169287	lanatus	Turkey, Bursa	2.7	1.1	4	0-6	3	0	0	0
PI 357693	lanatus	Macedonia	2.7	1.5	2	3-4	0	0	0	1
PI 476324	lanatus	Soviet Union, Former	2.7	1.5	2	1-4	1	0	0	0
PI 612471	lanatus	Korea, South, Pusan	2.7	1	5	1-4	3	1	1	2
PI 556994	lanatus	U.S., Alabama	2.7	1.5	2	1-2	2	1	1	1
PI 482283	amarus	Zimbabwe	2.8	1	5	0-6	2	3	1	1
PI 207473	lanatus	Afghanistan, Kabul	2.8	1	5	1-6	3	1	1	1
PI 254740	mucosospermus	Senegal	2.8	0.9	6	1-5	3	3	1	1
PI 593356	lanatus	China, Shaanxi	2.8	1.2	3	0-4	2	0	0	0
PI 200732	lanatus	El Salvador	2.8	1	5	1-5	3	3	0	0
Mickylee	lanatus	NA	2.8	0.6	23	0-7	10	3	2	4
PI 314655	lanatus	Uzbekistan	2.8	1	5	1-5	3	2	2	1
PI 381709	lanatus	India	2.8	1.1	4	0-6.2	2	0	0	0
PI 254741	mucosospermus	Senegal	2.8	0.8	8	0.5-4.5	3	3	2	2
PI 632754	lanatus	Bulgaria	2.8	1.3	3	0-7	2	1	1	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 275631	lanatus	India, Rajasthan	2.8	1.3	3	1-7	2	0	1	1
PI 512348	lanatus	Spain, Tarragona	2.8	1.1	4	1-6	3	0	0	0
PI 482254	lanatus	Zimbabwe	2.8	1	5	1-5	3	1	1	1
PI 593386	lanatus	China, Xinjiang	2.8	1.1	4	0.5-5	2	0	1	1
PI 525095	lanatus	Egypt, Sinai	2.8	1	5	1-5	2	3	3	2
PI 162667	lanatus	Argentina, Buenos Aires	2.8	1.1	4	0-6.5	1	2	1	1
PI 222713	lanatus	Iran, Bakhtaran	2.8	1.1	4	0.5-6	2	1	3	0
PI 507859	lanatus	Hungary	2.8	1.5	2	2-4	1	1	1	0
PI 476326	lanatus	Soviet Union, Former	2.8	1.1	4	1-4	2	0	0	0
PI 270546	lanatus	Ghana, Capital District	2.8	1.1	4	0-8	3	1	1	1
PI 270143	lanatus	India, Delhi	2.8	0.9	8	0-6	2	1	1	3
PI 459075	lanatus	Botswana	2.8	1.2	3	1-3	1	1	1	1
PI 174103	lanatus	Turkey, Mardin	2.8	1.5	2	3-3	0	1	0	0
PI 172793	lanatus	Turkey, Van	2.8	1.3	3	0-5	1	0	0	0
PI 379228	lanatus	Macedonia	2.8	1.3	3	0-5	1	1	0	0
Grif 5597	lanatus	India	2.8	1.1	4	1-5	2	1	2	1
PI 525099	lanatus	Egypt, Matruh	2.8	1.5	2	0.5-2.7	1	1	0	0
PI 357689	lanatus	Macedonia	2.8	0.9	6	0-9	4	1	1	3
PI 269677	lanatus	Belize	2.8	1.5	2	0-4	1	0	0	1
PI 482364	lanatus	Zimbabwe	2.8	0.8	7	0-7	5	1	2	1
PI 279459	lanatus	Japan	2.8	1.1	4	0-8	3	1	0	1
PI 482267	lanatus	Zimbabwe	2.8	1	5	0-8	3	2	2	1
PI 542616	colocynthis	Algeria	2.8	1.3	3	1.8-3.7	1	2	3	1
PI 279456	lanatus	Japan	2.8	1.1	4	0-5	2	0	0	1
PI 277994	lanatus	Turkey, Bilecik	2.8	1.5	2	3-3.7	0	1	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 278061	lanatus	Turkey, Yozgat	2.8	1.1	4	1-4.5	2	2	1	1
PI 560023	mucosospermus	Nigeria, Oyo	2.8	1	5	2-4	1	0	0	1
PI 379236	lanatus	Macedonia	2.9	1.1	4	1-4	2	2	0	0
PI 506439	lanatus	Moldova	2.9	1	5	1-6	2	1	3	2
PI 277975	lanatus	Turkey, Adiyaman	2.9	1	5	0-7	2	1	1	2
PI 357688	lanatus	Macedonia	2.9	0.9	6	0.5-5	3	2	2	2
PI 635682	lanatus	Argentina	2.9	0.9	6	1-8	4	0	1	2
PI 482274	lanatus	Zimbabwe	2.9	1	5	1-5.5	3	1	3	1
PI 177324	lanatus	Turkey, Corum	2.9	1.3	3	1-3	1	0	1	0
PI 278022	lanatus	Turkey, Kars	2.9	1.5	2	2-3	1	1	1	1
PI 357679	lanatus	Macedonia	2.9	1.3	3	2-4	1	1	1	0
PI 500342	lanatus	Zambia	2.9	1.5	2	1.5-4	1	0	0	0
PI 307609	lanatus	Nigeria	2.9	1.2	3	2.5-4	0	1	1	1
PI 357739	lanatus	Macedonia	2.9	0.9	8	0-9	4	1	0	2
PI 169292	lanatus	Turkey, Bursa	2.9	1.2	3	0-5	1	0	1	1
PI 482363	lanatus	Zimbabwe	2.9	1	5	1-5	3	2	3	3
PI 635598	lanatus	U.S., California	2.9	1.5	2	1-5	1	1	1	1
PI 512376	lanatus	Spain, Alicante	2.9	1.1	4	0.5-6	2	1	1	0
PI 629107	lanatus	U.S., California	2.9	1.5	2	2-2.5	1	0	0	0
PI 276658	lanatus	Russian Federation	2.9	1.2	3	1-4	1	1	0	1
PI 254743	mucosospermus	Senegal	2.9	1.1	4	1-3	1	1	1	0
PI 345546	lanatus	Soviet Union, Former	2.9	0.9	6	0-8	3	1	3	1
PI 494531	mucosospermus	Nigeria, Oyo	2.9	0.8	7	1.3-3.9	2	3	1	1
PI 635657	lanatus	U.S., Florida	2.9	1	6	0.5-7.3	3	3	0	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 176487	lanatus	Turkey, Tunceli	2.9	1	5	0-6	3	2	3	1
PI 270563	amarus	South Africa	2.9	1	5	1-6	2	1	0	0
PI 457916	mucosospermus	Liberia	2.9	0.9	7	0.5-6	4	1	1	2
PI 177330	lanatus	Syria	2.9	1.1	4	0-4	2	1	1	1
PI 233556	lanatus	Japan	2.9	0.9	6	1-6	3	1	2	1
Dixielee	lanatus	NA	2.9	0.8	8	0-8	5	1	0	1
PI 560016	mucosospermus	Nigeria, Oyo	2.9	0.9	6	0-6	3	2	2	1
PI 172792	lanatus	Turkey, Kars	2.9	1.3	3	1-3	1	0	1	0
PI 600951	lanatus	U.S.	2.9	1.3	3	3-4	0	1	1	1
PI 174109	lanatus	Turkey, Elazig	2.9	0.9	6	2-4	1	1	3	1
PI 165024	lanatus	Turkey, Ankara	2.9	0.9	6	0-4	3	0	0	2
PI 357733	lanatus	Macedonia	2.9	0.8	7	0-5	3	1	1	2
PI 193964	lanatus	Ethiopia	2.9	1.1	4	3-4.3	0	1	1	1
PI 183300	lanatus	India, Madhya Pradesh	2.9	0.9	6	0-7	4	2	2	1
PI 635631	lanatus	U.S., Georgia	2.9	1	6	2-4	2	1	2	0
PI 487476	lanatus	Israel	2.9	1.1	4	0-4	1	0	0	0
PI 184800	mucosospermus	Nigeria	2.9	0.9	8	1-5	2	1	2	2
PI 543209	lanatus	Bolivia, Beni	2.9	1	5	0.5-6	3	1	2	0
PI 244017	amarus	South Africa, Transvaal	2.9	0.8	7	1-5.5	3	5	2	2
PI 219691	lanatus	Pakistan	2.9	0.9	6	0-6	3	1	2	3
PI 381698	lanatus	India	2.9	1.1	4	0-4.5	1	1	1	1
PI 255136	amarus	South Africa, Transvaal	2.9	1.1	4	0.2-6	2	1	1	0
PI 169258	lanatus	Turkey, Manisa	3	0.9	8	0-7	3	0	0	0
PI 500304	lanatus	Zambia	3	1	5	1-4.3	2	2	2	1
PI 593378	lanatus	China, Xinjiang	3	0.9	6	0-7	3	1	0	1
PI 169283	lanatus	Turkey, Balikesir	3	1.1	4	0-4	1	0	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 222711	lanatus	Iran, West Azerbaijan	3	0.9	9	1-5	3	2	3	4
PI 381703	lanatus	India	3	0.7	15	0-9	8	2	1	6
PI 179880	lanatus	India, Rajasthan	3	1	5	1.5-5	3	0	1	1
PI 512402	lanatus	Spain, Cadiz	3	1.3	3	1-3	1	1	2	0
PI 269466	lanatus	Pakistan, Northern Areas	3	1.1	4	0-5	2	1	1	2
PI 227206	lanatus	Japan, Aichi	3	1.3	3	1-4	1	2	1	2
PI 612472	lanatus	Korea, South, Pusan	3	1	5	0-5	2	1	1	1
PI 593347	lanatus	China, Henan	3	1.1	4	0-5	2	0	0	0
PI 248774	amarus	Namibia	3	1.1	4	0-5	1	2	1	1
PI 169260	lanatus	Turkey, Manisa	3	1.1	4	0-3	1	1	2	1
PI 246559	lanatus	Senegal	3	1	5	1-5	2	1	3	0
PI 537267	lanatus	Pakistan, Punjab	3	1.1	4	1-5	1	4	2	2
PI 169248	lanatus	Turkey, Mugla	3	1.1	4	1-7	2	2	2	1
PI 276659	lanatus	Russian Federation	3	1	5	0-5.2	3	1	1	1
PI 532732	mucosospermus	Zaire, Bandundu	3	0.9	6	1-7	2	2	0	1
PI 482281	lanatus	Zimbabwe	3	0.9	6	0.7-7	3	2	2	1
PI 181744	lanatus	Lebanon	3	1.1	4	1-6.5	2	0	1	1
PI 658680	lanatus	China, Jiangsu	3	1	7	0-6	2	0	1	1
PI 357661	lanatus	Macedonia	3	1.3	3	1-4	1	1	1	1
PI 175664	lanatus	Turkey, Kayseri	3	1.3	3	3-4	0	1	1	1
PI 482378	lanatus	Zimbabwe	3	1	5	1-6	2	2	3	0
PI 169280	lanatus	Turkey, Balikesir	3	1.1	4	0-4	2	2	3	1
PI 357684	lanatus	Macedonia	3	1.5	2	1-4	1	1	1	1
PI 537275	lanatus	Pakistan, Punjab	3	0.9	6	0-5.3	3	2	2	2
PI 560002	mucosospermus	Nigeria, Oyo	3	0.9	6	0-9	5	3	3	0
PI 183126	lanatus	India, Maharashtra	3	1.1	4	0-7	3	1	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 178873	lanatus	Turkey, Cankiri	3	1.1	6	0.5-7	3	1	2	4
PI 183123	lanatus	India, Gujarat	3	0.9	13	0-8	7	3	1	4
PI 357730	lanatus	Macedonia	3	1.2	3	0-4	1	0	1	0
PI 629103	lanatus	India, Rajasthan	3	0.9	6	0-6.5	3	2	1	2
PI 381694	lanatus	India	3	1.1	4	0-4	2	1	0	0
PI 560003	mucosospermus	Nigeria, Oyo	3	0.9	6	1-5.3	1	2	2	1
PI 169259	lanatus	Turkey, Manisa	3	1	5	0.5-4	2	2	1	1
PI 278006	lanatus	Turkey, Gaziantep	3	0.8	8	0-6	4	3	1	2
PI 612457	lanatus	Korea, South, Pusan	3.1	1.5	2	1-4	1	0	0	0
PI 180278	lanatus	India, Gujarat	3.1	1.3	4	2.5-5	0	0	1	1
PI 512359	lanatus	Spain, Caceres	3.1	1.1	4	1.5-3.5	2	2	3	2
PI 629101	lanatus	NA	3.1	1.5	2	2-3	1	0	1	1
PI 381708	lanatus	India	3.1	1.1	4	1.7-5	1	1	1	0
PI 176915	lanatus	Turkey, Konya	3.1	1.5	2	1-4	1	0	0	0
PI 525080	colocynthis	Egypt, Qena	3.1	1.5	2	1-6	1	0	1	1
PI 518609	lanatus	Soviet Union, Former	3.1	1.3	3	0-6.5	1	0	1	1
PI 164636	lanatus	India, Karnataka	3.1	1	5	1-6	2	1	1	1
Jubilee	lanatus	NA	3.1	1	5	0-6	2	1	2	1
PI 178871	lanatus	Turkey, Erzincan	3.1	1.3	3	2.7-4	0	1	1	2
PI 174106	lanatus	Turkey, Gaziantep	3.1	1	5	0-6	3	1	1	1
PI 512358	lanatus	Spain, Caceres	3.1	0.9	6	1-4	3	2	2	1
PI 368529	lanatus	Macedonia	3.1	1	5	1-6	3	2	2	1
PI 534589	lanatus	Syria	3.1	1.1	4	2-4	1	3	2	1
PI 560014	mucosospermus	Nigeria, Ogun	3.1	0.8	8	0-5	3	2	3	0
PI 601182	lanatus	U.S.	3.1	1.5	2	1-3.3	1	2	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 512346	lanatus	Spain, Tarragona	3.1	1.5	2	3-3	0	0	0	1
PI 169273	lanatus	Turkey, Kirklareli	3.1	1.3	3	0-4.5	1	0	1	1
PI 482372	lanatus	Zimbabwe	3.1	1	5	0-5	3	1	1	0
PI 635709	lanatus	Australia, Queensland	3.1	1.1	4	1-6.5	2	1	1	0
PI 278003	lanatus	Turkey, Bursa	3.1	1.1	4	2.5-3	0	2	3	2
PI 357732	lanatus	Macedonia	3.1	1.5	2	0-4.5	1	1	1	0
PI 176493	lanatus	Turkey, Nigde	3.1	1.1	4	1-4.5	2	0	2	1
PI 494529	mucosospermus	Nigeria, Oyo	3.1	0.9	6	0-7	3	3	2	1
PI 593361	lanatus	China, Xinjiang	3.1	1	5	1-5	2	2	0	2
PI 505591	lanatus	Zambia	3.1	0.9	6	1-5	1	0	0	2
PI 278000	lanatus	Turkey, Burdur	3.1	1.1	4	1-8	3	2	2	2
PI 635662	lanatus	U.S., California	3.1	1	5	0-7	2	1	1	0
PI 458738	lanatus	Paraguay, Chaco	3.1	1.5	2	1-6	1	0	0	1
PI 175659	lanatus	Turkey, Kirsehir	3.1	1.5	2	1-5	1	1	0	0
PI 179661	lanatus	India, Rajasthan	3.1	1.2	3	2.5-3	0	1	1	0
PI 210017	lanatus	India, Assam	3.1	1.5	2	2-4	1	0	0	0
PI 635722	lanatus	U.S., Georgia	3.2	1.1	4	1.7-5	1	1	2	2
PI 278024	lanatus	Turkey, Kayseri	3.2	1.5	2	1-4.5	1	2	1	1
PI 512407	lanatus	Spain, Cadiz	3.2	1	5	1-5	2	0	0	2
PI 179240	lanatus	Turkey, Edirne	3.2	0.9	6	0-4.5	2	1	1	1
Quetzali	lanatus	NA	3.2	1.2	3	1-7.3	1	1	1	1
PI 370432	lanatus	Macedonia	3.2	0.8	7	1-5	3	4	4	2
PI 512382	lanatus	Spain, Castellon de Plana	3.2	1	5	0.5-6	2	2	0	1
PI 169237	lanatus	Turkey, Izmir	3.2	1.3	3	1-6	2	0	0	1
PI 278025	lanatus	Turkey, Kirklareli	3.2	1	5	0-6	2	1	2	1
PI 482336	amarus	Zimbabwe	3.2	1.3	3	0-8	1	1	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 635755	lanatus	U.S., Maryland	3.2	1.5	2	0.2-5	1	0	0	0
PI 475746	lanatus	Paraguay, Misiones	3.2	1.3	3	1-4	2	1	1	1
PI 482329	lanatus	Zimbabwe	3.2	1.1	4	0-5	1	2	3	0
PI 593365	lanatus	China, Xinjiang	3.2	1.3	3	2-4	1	0	1	1
PI 593348	lanatus	China, Henan	3.2	1.1	4	0.5-5	2	0	1	1
PI 278019	lanatus	Turkey, Izmir	3.2	1	5	1-8	2	0	1	0
PI 635665	lanatus	U.S., South Carolina	3.2	1.5	2	0-4	1	1	0	0
PI 176498	lanatus	Turkey, Eskisehir	3.2	1.5	2	1-3	1	1	1	0
PI 278021	lanatus	Turkey, Kars	3.2	1	5	1.5-8	4	1	1	3
PI 482318	lanatus	Zimbabwe	3.2	1.5	2	2-4	1	1	1	1
PI 274794	lanatus	Pakistan	3.2	1.1	4	1-5	2	2	1	1
PI 512365	lanatus	Spain, Caceres	3.2	1.2	3	1-4	1	0	1	1
PI 169265	lanatus	Turkey, Istanbul	3.2	1.1	4	1-6	2	0	1	1
PI 295848	lanatus	South Africa	3.2	1.2	3	2-5	1	0	1	0
PI 179878	lanatus	India, Rajasthan	3.2	1	5	0-6	2	1	1	0
PI 482339	lanatus	Zimbabwe	3.2	1.1	4	0.5-7	2	1	1	1
PI 512392	lanatus	Spain, Castellon de Plana	3.2	1	5	0-5	3	1	0	2
PI 635730	lanatus	U.S., Florida	3.2	1.1	4	1-6	2	1	1	0
PI 163574	lanatus	Guatemala, Jutiapa	3.2	1.5	2	1.3-5	1	0	0	1
PI 381717	lanatus	India	3.2	0.8	13	0.5-8	5	3	4	4
PI 381733	lanatus	India	3.2	1.1	4	0-5.5	2	0	0	1
PI 165448	lanatus	Mexico, Oaxaca	3.2	1	5	0-5	2	1	0	0
PI 518612	lanatus	Soviet Union, Former	3.2	1	6	0-8	3	0	0	2
PI 505584	lanatus	Zambia	3.2	1	5	0-5	2	2	1	0
PI 195927	colocynthis	Ethiopia	3.2	0.8	7	1-9	5	1	1	1
PI 169257	lanatus	Turkey, Manisa	3.3	1	5	1-5	3	2	2	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 534595	lanatus	Syria	3.3	1.3	3	0-5.3	1	1	2	1
PI 254623	lanatus	Sudan, Khartoum	3.3	0.9	7	1.5-9	3	0	1	1
PI 178876	lanatus	Turkey, Bursa	3.3	0.9	6	2-4	2	1	1	0
PI 169284	lanatus	Turkey, Balikesir	3.3	1.1	4	1-6	2	2	1	1
PI 306367	lanatus	Angola	3.3	1.5	2	0-6	1	1	1	0
PI 482263	lanatus	Zimbabwe	3.3	1	5	0-7	2	0	1	0
PI 220779	lanatus	Afghanistan	3.3	1.3	4	2-6	1	1	3	2
PI 508446	lanatus	Korea, South	3.3	1.1	4	1-4	1	1	2	0
PI 381697	lanatus	India	3.3	0.8	7	0-6	2	0	0	1
PI 270144	lanatus	Greece	3.3	1	5	0.5-7	2	1	3	1
PI 482319	amarus	Zimbabwe	3.3	1.2	4	0-7	2	0	0	0
PI 482366	lanatus	Zimbabwe	3.3	0.9	7	0-9	4	0	2	3
PI 200733	lanatus	Guatemala, Alta Verapaz	3.3	1	5	2-5	2	1	1	0
PI 278030	lanatus	Turkey, Kirsehir	3.3	1.1	4	2-4.8	1	0	1	0
PI 176916	lanatus	Turkey, Konya	3.3	1.2	3	1-7	2	0	0	0
PI 379223	lanatus	Serbia	3.3	1.1	4	0-5	2	1	1	1
PI 357659	lanatus	Macedonia	3.3	0.9	6	0.8-9	3	1	1	1
PI 177326	lanatus	Turkey, Hakkari	3.3	0.8	13	0-9	5	1	1	1
PI 225557	lanatus	Zimbabwe	3.3	0.8	7	1.5-8	3	4	3	3
PI 482315	amarus	Zimbabwe	3.3	1.5	2	1-5	1	1	1	1
PI 186974	lanatus	Ghana	3.3	1	5	1-7	2	1	1	2
PI 593351	lanatus	China, Henan	3.3	1	5	1-7	3	2	1	1
PI 171580	lanatus	Turkey, Samsun	3.3	0.9	6	0.7-5	3	1	2	2
PI 176491	lanatus	Turkey, Sivas	3.3	1	7	2-5.2	1	1	4	4
PI 167126	lanatus	Turkey, Adana	3.3	1.1	4	1-4	1	1	1	1
PI 593384	lanatus	China, Xinjiang	3.3	1.2	3	0-8	2	2	1	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 203551	lanatus	U.S., New Mexico	3.3	0.8	7	1-9	4	2	2	3
PI 164634	lanatus	India, Karnataka	3.4	1.2	3	2-3	2	1	1	0
PI 534585	lanatus	Syria	3.4	1.3	3	0-7	1	2	0	0
PI 222714	lanatus	Iran, Bakhtaran	3.4	1.1	4	0-7	1	1	2	1
PI 482337	lanatus	Zimbabwe	3.4	1.2	5	1-5	2	0	2	1
PI 537276	lanatus	Pakistan, Punjab	3.4	0.8	7	0-6	2	0	2	0
PI 381705	lanatus	India	3.4	1	5	0-6	2	3	2	1
PI 172798	lanatus	Turkey, Mardin	3.4	1.3	3	2-5	1	1	1	1
PI 635616	lanatus	U.S., New York	3.4	1	5	1.5-7	2	2	2	1
PI 381723	lanatus	India	3.4	1.3	3	4-4	0	0	0	0
PI 229605	lanatus	Iran, Mazandaran	3.4	1.5	2	2.5-4.5	0	0	1	1
PI 532817	lanatus	China	3.4	0.9	6	1-6	3	3	3	3
PI 175656	lanatus	Turkey, Urfa	3.4	1	5	0-6	1	0	0	0
PI 379248	lanatus	Macedonia	3.4	1.1	4	2-7	2	0	0	1
PI 370015	lanatus	India	3.4	1.3	3	0-9	2	0	0	1
PI 277974	lanatus	Turkey, Adiyaman	3.4	1	5	1-9	4	1	2	1
PI 171585	lanatus	Turkey, Tokat	3.4	1.2	4	2.5-5	0	0	0	2
PI 378613	lanatus	Zaire	3.4	1	5	0-8	2	0	0	0
PI 173670	lanatus	Turkey, Urfa	3.4	1.5	2	2.3-5	0	0	0	1
PI 169295	lanatus	Turkey, Bursa	3.4	1.1	4	1-5.5	1	0	1	2
PI 560004	mucosospermus	Nigeria, Oyo	3.4	1	5	1-5.5	2	0	0	0
PI 270309	lanatus	Philippines	3.4	0.9	6	0-7	3	2	2	2
PI 512379	lanatus	Spain, Valencia	3.4	1.2	3	1-5	1	1	0	0
PI 171579	lanatus	Turkey, Zonguldak	3.4	0.9	6	0-6.2	3	1	1	3
PI 500314	lanatus	Zambia	3.4	0.9	6	0.5-7	2	2	2	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 269680	lanatus	Belize	3.4	1.5	2	2-6	1	0	0	0
PI 379234	lanatus	Macedonia	3.4	1.3	3	2-4	1	1	1	1
PI 214044	lanatus	India, Karnataka	3.4	1.1	4	0-5	2	0	0	2
PI 507865	lanatus	Hungary, Szabolcs-Szatmar	3.4	1	5	0-9	1	1	1	2
Grif 5595	lanatus	India	3.4	0.9	6	1-6	2	2	1	3
PI 182181	lanatus	Turkey, Balikesir	3.4	1	5	2.5-5	0	1	2	1
PI 357678	lanatus	Macedonia	3.4	1.1	4	0-5	1	0	0	1
PI 512833	lanatus	Spain, Castellon de Plana	3.4	1	5	0-5	2	1	1	1
PI 306365	lanatus	Gabon	3.4	1.1	4	0.7-9	3	1	2	1
PI 186975	mucosospermus	Ghana	3.5	1	5	1-8	2	1	0	0
PI 277978	lanatus	Turkey, Afyon	3.5	0.9	6	0-8	2	3	2	2
PI 357737	lanatus	Macedonia	3.5	1.1	4	1-7	1	1	1	3
PI 288522	lanatus	India, Gujarat	3.5	0.8	8	1-8	5	4	3	2
PI 534532	lanatus	Syria	3.5	1.2	3	0-6	1	0	1	0
PI 175662	lanatus	Turkey, Kayseri	3.5	1.5	2	2-4	1	1	1	0
PI 500302	lanatus	Zambia	3.5	1.2	4	0.3-7	2	1	1	1
PI 171583	lanatus	Turkey, Tokat	3.5	1.3	3	2-5.5	1	1	1	1
PI 270549	lanatus	Ghana	3.5	1.1	4	2-4	1	1	2	2
PI 368518	lanatus	Macedonia	3.5	1.3	3	1-7	2	0	0	1
PI 536449	lanatus	Maldives	3.5	1.1	4	2.2-5	0	1	2	1
PI 164998	lanatus	Turkey, Ankara	3.5	1.1	4	0-8	2	2	0	1
PI 177329	lanatus	Turkey, Hakkari	3.5	1.3	3	2-4	1	1	0	1
PI 177325	lanatus	Turkey, Hakkari	3.5	1.3	3	2-4	1	2	0	1
PI 536453	lanatus	Maldives	3.5	1.1	4	2-5	1	0	0	3
PI 174098	lanatus	Turkey, Corum	3.5	1.5	2	2-5	1	1	1	0
PI 482380	lanatus	Zimbabwe	3.5	0.8	9	0-9	4	2	2	3

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 357726	lanatus	Macedonia	3.5	1.1	4	0-7	2	1	1	1
PI 525098	lanatus	Egypt, Giza	3.5	1.1	4	1-7	2	1	1	0
PI 113326	lanatus	China	3.5	1.5	2	0-7	1	1	1	1
PI 278031	lanatus	Turkey, Kirsehir	3.5	1	5	1-9	2	0	0	3
PI 665007	lanatus	U.S., Colorado	3.5	1	5	1-7	3	2	3	1
PI 211917	lanatus	NA	3.5	1.5	2	1-4	1	1	0	1
PI 595201	lanatus	U.S., Georgia	3.5	1.1	4	0.5-5	1	1	2	0
PI 487459	lanatus	Venezuela, Amazonas	3.5	1	5	0-8	3	1	2	2
PI 278054	lanatus	Turkey, Tunceli	3.5	1.3	3	0-5.5	1	1	1	1
PI 379239	lanatus	Macedonia	3.5	1.1	4	1-6	2	3	2	2
PI 277999	lanatus	Turkey, Bolu	3.5	1.3	4	1-7	1	0	0	0
PI 482341	lanatus	Zimbabwe	3.5	1.3	3	0-5.5	1	1	1	1
PI 278026	lanatus	Turkey, Kirklareli	3.5	1.1	4	1-6	1	2	1	1
PI 537468	lanatus	Spain, Gerona	3.5	1.1	4	0-9	3	1	2	1
PI 169285	lanatus	Turkey, Balikesir	3.5	1.1	4	1-7	1	2	2	2
PI 270551	lanatus	Ghana	3.5	1.3	3	0.5-6.5	1	1	0	1
PI 561041	lanatus	U.S.	3.5	1.2	3	2-5.5	2	2	0	0
PI 254624	lanatus	Sudan, Khartoum	3.5	1.1	4	0.2-7	1	0	0	0
PI 271776	lanatus	South Africa, Transvaal	3.5	1	5	1-9	2	0	1	3
PI 512356	lanatus	Spain, Toledo	3.6	0.8	15	0-7	4	2	3	2
PI 500337	lanatus	Zambia	3.6	1.3	3	2-5	1	1	1	2
PI 378614	lanatus	Zaire	3.6	1.3	3	2-6	1	0	0	0
PI 379240	lanatus	Macedonia	3.6	1.1	4	1-6.5	1	1	1	1
PI 482376	lanatus	Zimbabwe	3.6	1.1	4	1.7-5	2	1	1	2
PI 172787	lanatus	Turkey, Trabzon	3.6	1.3	3	0-6	2	2	2	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 277973	lanatus	Turkey, Adiyaman	3.6	1.3	3	1.5-5	1	1	1	0
PI 221430	lanatus	Iran	3.6	1	5	1.5-6.5	2	2	3	1
PI 357716	lanatus	Macedonia	3.6	0.9	6	1-8	4	1	2	1
PI 482268	lanatus	Zimbabwe	3.6	1.1	4	1-7	1	3	2	1
PI 169261	lanatus	Turkey, Manisa	3.6	1.3	3	1-6.5	1	0	0	0
PI 271752	lanatus	Ghana, Central	3.6	1.1	4	1-5	1	0	0	0
PI 482306	lanatus	Zimbabwe	3.6	0.9	6	1.5-8	2	3	4	2
PI 182179	lanatus	Turkey, Mardin	3.6	1.3	3	1-5	1	0	0	1
PI 612463	lanatus	Korea, South, Pusan	3.6	1	5	2-6.5	2	1	0	3
PI 161373	lanatus	Korea, South, Kyonggi	3.6	1	5	1-6	1	1	3	2
PI 635714	lanatus	U.S., California	3.6	1.1	4	1-7	2	0	0	1
PI 271983	lanatus	Somalia	3.6	1.4	3	3-7	0	0	2	1
PI 278001	lanatus	Turkey, Bursa	3.6	1.5	2	2.5-4	0	0	0	0
PI 278009	lanatus	Turkey, Gaziantep	3.6	1	5	1-7	1	2	2	3
PI 368494	lanatus	Macedonia	3.6	1.5	2	3-5	0	0	1	0
PI 381740	lanatus	India	3.6	0.9	6	0-8	4	1	1	2
PI 525100	lanatus	Egypt, Cairo	3.6	1.1	4	2-5.2	2	1	3	1
PI 357711	lanatus	Macedonia	3.6	1.3	3	0-6	1	1	1	0
PI 278057	lanatus	Turkey, Urfa	3.6	1.4	3	1-5	1	0	2	1
PI 278002	lanatus	Turkey, Bursa	3.6	1	5	1-7	2	1	1	1
PI 482381	lanatus	Zimbabwe	3.6	0.9	6	0-9	3	1	1	1
PI 612470	lanatus	Korea, South, Pusan	3.6	1.3	3	1-7	2	1	0	1
PI 357708	lanatus	Macedonia	3.6	1.1	4	2-8	2	1	2	0
PI 163203	lanatus	India, Punjab	3.6	1.5	2	3-4	0	1	1	0
PI 482269	lanatus	Zimbabwe	3.6	1.1	4	3.5-4.5	0	1	1	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 169276	lanatus	Turkey, Canakkale	3.6	0.9	6	1-5	2	2	0	1
PI 536459	lanatus	Maldives	3.6	1.2	4	3-6	0	1	0	1
PI 223764	lanatus	Afghanistan, Badakhshan	3.6	1.2	3	0-8	2	2	1	1
PI 368493	lanatus	Macedonia	3.6	1	5	0-5	1	0	0	0
PI 222137	lanatus	Algeria, Oran	3.6	1.3	3	1-6.3	1	1	1	1
PI 180426	lanatus	India, Madhya Pradesh	3.6	0.9	6	1.5-6	3	3	2	2
PI 176912	lanatus	Turkey, Konya	3.6	1.1	4	0-7	2	1	0	1
PI 379247	lanatus	Macedonia	3.6	1	5	1-8	2	0	0	2
PI 271750	lanatus	Ghana, Upper	3.6	1.3	3	2-5	2	1	0	0
PI 381715	lanatus	India	3.6	1.1	4	1-5	1	0	0	0
PI 532811	lanatus	China	3.6	1.5	2	2-5	1	0	1	0
PI 476325	lanatus	Ukraine	3.6	1.5	2	3-4	0	0	1	0
PI 612462	lanatus	Korea, South, Pusan	3.6	0.8	13	0-9	5	1	0	6
PI 502316	lanatus	Uzbekistan, Samarqand	3.7	1.5	2	3-3	0	0	0	0
PI 482370	lanatus	Zimbabwe	3.7	1	5	1-9	3	1	1	2
PI 526237	lanatus	Zimbabwe	3.7	0.9	6	1-9	2	1	3	2
PI 482374	lanatus	Zimbabwe	3.7	1.1	4	3-5	0	1	2	1
PI 176499	lanatus	Turkey, Eskisehir	3.7	0.8	15	1-8.5	6	2	1	1
PI 635601	lanatus	U.S., California	3.7	1.1	4	2-5	1	0	0	0
PI 379249	lanatus	Macedonia	3.7	1.1	4	0-6.5	1	1	1	0
PI 234287	lanatus	Portugal, Lisboa	3.7	1.2	3	2-5	2	0	1	0
PI 476330	lanatus	Soviet Union, Former	3.7	0.8	8	1-9	5	0	1	2
PI 512355	lanatus	Spain, Toledo	3.7	1	5	1-9	3	0	2	2
PI 560012	mucosospermus	Nigeria, Ogun	3.7	0.9	6	0-6	3	3	2	0
PI 357686	lanatus	Macedonia	3.7	1	5	0-9	3	0	0	1
PI 271987	lanatus	Somalia	3.7	1.3	3	0-8	1	1	2	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 183299	lanatus	India, Madhya Pradesh	3.7	0.9	7	0.5-8	3	1	1	4
PI 381716	lanatus	India	3.7	1.1	4	2-5	1	1	0	1
PI 534587	lanatus	Syria	3.7	1.3	3	0-6	1	0	0	0
PI 274561	lanatus	Portugal	3.7	1.3	3	1.5-5.2	2	1	2	2
PI 270547	lanatus	Ghana	3.7	1.5	2	1-6	1	2	2	2
PI 512351	lanatus	Spain, Toledo	3.7	1.3	4	1-6.7	2	0	1	2
PI 593377	lanatus	China, Xinjiang	3.7	0.9	6	0-7	3	1	1	2
PI 357722	lanatus	Macedonia	3.7	1.5	2	1-5	1	1	1	1
PI 368514	lanatus	Macedonia	3.7	0.9	6	1-6	2	1	2	0
PI 357675	lanatus	Macedonia	3.7	1.3	3	1-5	1	0	0	0
PI 278044	lanatus	Turkey, Mugla	3.7	0.8	8	0-8	3	1	0	3
PI 181937	lanatus	Syria	3.7	1.1	4	1-5	2	0	1	2
PI 212094	lanatus	Afghanistan, Ghazni	3.7	1.5	2	3-6	0	0	0	1
PI 381712	lanatus	India	3.7	0.9	6	1-9	3	1	2	4
PI 277988	lanatus	Turkey, Aydin	3.7	0.9	11	0-8	7	2	1	1
PI 512370	lanatus	Spain, Alicante	3.7	1.3	3	1-4	1	0	0	1
PI 476328	lanatus	Soviet Union, Former	3.7	1.5	2	3-4	0	0	1	0
PI 179236	lanatus	Turkey, Tekirdag	3.7	1.5	2	2-4.2	1	1	0	1
PI 219906	lanatus	Afghanistan	3.7	1.2	3	0-5.5	1	0	0	0
PI 164709	lanatus	India, Karnataka	3.7	0.9	9	1-8	2	2	2	3
PI 179884	lanatus	India, Gujarat	3.7	1	5	1-9	2	1	0	2
PI 381707	lanatus	India	3.8	1.1	4	2-6	1	1	1	1
PI 512342	lanatus	Spain, Zaragoza	3.8	1.1	4	0-9	1	0	0	1
PI 176496	lanatus	Turkey	3.8	1.5	2	3.3-3.5	0	0	0	1
PI 635612	lanatus	U.S., Minnesota	3.8	1.5	2	3.5-5	0	1	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 175657	lanatus	Turkey, Urfa	3.8	1.2	3	1.5-8	2	1	2	2
PI 512369	lanatus	Spain, Valencia	3.8	1	7	1-9	3	0	1	2
Des. King	lanatus	NA	3.8	0.8	11	1-7	5	0	3	2
PI 379226	lanatus	Macedonia	3.8	1.3	3	2-4	1	1	1	1
PI 500307	lanatus	Zambia	3.8	1	5	2-7	1	2	4	1
Starbrite	lanatus	NA	3.8	0.7	13	0-8	3	3	4	3
PI 269678	lanatus	Belize	3.8	1.3	3	1-8	2	0	2	0
PI 507867	lanatus	Hungary	3.8	1.1	4	1-8	2	0	0	1
PI 526236	lanatus	Zimbabwe	3.8	1.1	4	0.8-8	1	1	1	1
Grif 14199	lanatus	India, Rajasthan	3.8	0.8	8	0-8	3	3	3	3
PI 629105	lanatus	U.S., North Carolina	3.8	1	5	1-6.7	2	3	3	2
PI 381711	lanatus	India	3.8	1.1	4	2-5.5	1	1	2	0
PI 534530	lanatus	Syria	3.8	1	5	2-6	2	1	1	3
PI 381714	lanatus	India	3.8	0.8	7	0-9	2	1	1	1
PI 227203	lanatus	Japan, Shizuoka	3.8	1.2	3	1-5	1	3	1	3
PI 500318	lanatus	Zambia	3.8	1	5	2.5-5.5	0	3	1	2
PI 182180	lanatus	Turkey, Maras	3.8	0.9	7	1-7	1	1	5	2
PI 535948	lanatus	Cameroon	3.8	1.5	2	1.5-5	1	0	1	1
PI 255139	lanatus	South Africa	3.8	1	5	0.5-6	1	1	3	1
PI 105445	lanatus	Turkey, Amasya	3.8	1.3	3	0-5	1	0	0	1
PI 464872	lanatus	China	3.8	1	5	1-7	2	1	1	2
PI 357685	lanatus	Macedonia	3.8	1.1	4	1-9	2	1	2	2
Fiesta	lanatus	NA	3.8	0.9	7	1-6	2	2	2	3
PI 593390	lanatus	China, Xinjiang	3.8	1	5	0-8	3	1	2	1
PI 526239	lanatus	Zimbabwe	3.8	1.5	2	3.2-4.7	0	1	1	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v] Blotching	Cracking	Necrosis
PI 512341	lanatus	Spain, Zaragoza	3.8	1.5	2	1-5.5	1	0	1	0
PI 368500	lanatus	Macedonia	3.8	1	5	1-5	2	3	1	1
PI 279458	lanatus	Japan	3.8	1.1	4	2-5	1	1	3	3
PI 165451	lanatus	Mexico, Oaxaca	3.9	1.1	4	0-9	2	1	0	1
Regency	lanatus	NA	3.9	0.9	7	1-7	3	0	0	1
PI 171581	lanatus	Turkey, Tokat	3.9	1.2	3	0-5.5	1	0	0	2
PI 537461	lanatus	Spain	3.9	1.5	2	2.5-5	0	1	1	0
PI 512401	lanatus	Spain, Cadiz	3.9	1	7	0-9	2	2	2	4
PI 525094	lanatus	Egypt, Sinai	3.9	1	5	1-6	2	3	2	2
PI 482321	amarus	Zimbabwe	3.9	1.3	3	1-6	1	1	1	1
PI 593349	lanatus	China, Henan	3.9	1.3	3	0-8	2	2	2	1
PI 278052	lanatus	Turkey, Sivas	3.9	1	5	0-9	2	0	0	2
PI 559992	mucosospermus	Nigeria, Ogun	3.9	0.9	6	2-6.5	1	1	2	1
PI 369220	lanatus	Soviet Union, Former	3.9	0.9	6	2-6	2	1	1	0
PI 534535	lanatus	Syria	3.9	1.1	4	1-7	1	0	1	0
PI 601228	lanatus	U.S.	3.9	0.9	6	1-7	2	1	1	2
PI 386021	colocynthis	Iran	3.9	1.3	3	1-8	1	2	1	1
PI 487458	lanatus	Venezuela, Amazonas	3.9	1.1	4	1-8	2	1	0	2
PI 560901	lanatus	China	3.9	1.3	3	1-5	1	0	1	0
PI 601307	lanatus	U.S., Florida	3.9	1	5	2-8	1	3	1	3
PI 269676	lanatus	Belize	3.9	1.3	3	2-5	1	0	1	1
PI 277995	lanatus	Turkey, Bilecik	3.9	1	5	2.5-5	0	2	2	2
PI 601382	lanatus	U.S., Louisiana	3.9	1.2	3	1-9	1	1	1	2
PI 518607	lanatus	Soviet Union, Former	3.9	1	5	0-9	3	1	1	3
PI 178870	lanatus	Turkey, Zonguldak	3.9	1.5	2	4-4	0	0	1	0
PI 167045	lanatus	Turkey, Hatay	3.9	1.1	4	1-6	1	1	0	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 507862	lanatus	Hungary	3.9	1.1	4	0-9	2	0	0	1
PI 177328	lanatus	Turkey, Hakkari	3.9	1.3	3	4-4.5	0	0	1	2
PI 175661	lanatus	Turkey, Kirsehir	3.9	0.9	6	2-5	1	3	2	0
PI 207472	lanatus	Afghanistan, Kabul	3.9	1.3	3	2-5	1	1	2	1
PI 635621	lanatus	U.S., California	3.9	1.1	4	0-7	1	1	2	0
PI 368509	lanatus	Macedonia	3.9	1.1	4	1-8	2	0	0	1
PI 370425	lanatus	Macedonia	3.9	1.5	2	3.5-4	0	0	1	0
PI 270548	lanatus	Ghana	4	1.1	4	0-6.5	1	1	1	2
PI 537472	lanatus	Spain, Alicante	4	1.5	2	0-6	1	1	0	1
PI 612467	lanatus	Korea, South, Pusan	4	1.1	4	1-7	2	0	0	1
PI 270565	lanatus	South Africa, Cape Province	4	0.9	6	1-7	2	3	2	1
PI 169270	lanatus	Turkey, Kirklareli	4	1.1	4	0.3-6	1	1	0	1
PI 543212	lanatus	Bolivia, Beni	4	1.3	3	1-7	1	1	1	2
PI 171582	lanatus	Turkey, Amasya	4	1	5	1-7.5	2	1	1	2
PI 212208	lanatus	Greece	4	1.5	2	2-7	1	0	0	0
PI 169293	lanatus	Turkey, Bursa	4	1.1	4	0-9	2	0	1	0
PI 512362	lanatus	Spain, Caceres	4	1.2	3	2-4	1	1	1	1
PI 370422	lanatus	Serbia	4	1.3	3	2-7	2	1	1	1
PI 596662	amarus	South Africa, Transvaal	4	0.8	11	0-8	4	4	1	0
PI 535947	lanatus	Cameroon	4	1	5	3-7	0	3	2	3
PI 512331	lanatus	China, Beijing	4	1.2	4	1-7.7	2	0	1	1
PI 482357	lanatus	Zimbabwe	4	1.1	4	1.3-9	2	0	0	1
PI 368508	lanatus	Macedonia	4	0.9	6	1-7	1	2	2	2
PI 536452	lanatus	Maldives	4	1.3	3	2.5-7	0	1	1	3
Grif 1734	lanatus	China, Jiangsu	4	1	5	1-6	2	2	1	1
PI 512353	lanatus	Spain, Toledo	4	1.3	3	0-6	1	1	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 278020	lanatus	Turkey, Izmir	4	1.1	4	2-6	1	2	0	2
PI 357680	lanatus	Macedonia	4	1.3	3	0-9	2	1	0	2
PI 357664	lanatus	Macedonia	4	1.1	4	0-8	1	1	1	2
PI 500350	lanatus	Zambia	4	1.1	4	1-8	1	2	2	1
PI 518610	lanatus	Soviet Union, Former	4	1.3	3	3-6.2	0	0	1	1
PI 470248	lanatus	Indonesia, Kalimantan	4	0.9	6	0-7	2	3	2	3
PI 179235	lanatus	Turkey, Samsun	4	0.9	6	0-9	3	0	0	2
PI 172796	lanatus	Turkey, Mardin	4.1	0.9	6	1-7	2	1	2	2
PI 172805	lanatus	Turkey, Malatya	4.1	1.1	4	2.5-7	0	1	2	1
PI 482334	amarus	Zimbabwe	4.1	0.9	6	2-9	1	2	2	1
PI 379233	lanatus	Macedonia	4.1	1.5	2	1-6	1	0	1	0
PI 176921	lanatus	Turkey, Manisa	4.1	1.2	3	3-4	0	2	2	1
PI 177322	lanatus	Turkey, Istanbul	4.1	1.1	4	1.7-9	3	0	1	1
PI 254737	mucosospermus	Senegal	4.1	1.1	4	0-6	1	2	1	2
PI 278028	lanatus	Turkey, Kirklareli	4.1	1.1	4	0-7	1	1	0	2
PI 537266	lanatus	Pakistan, Punjab	4.1	0.8	7	1-8	2	3	4	2
PI 370431	lanatus	Macedonia	4.1	1.5	2	1-8	1	1	1	1
PI 635614	lanatus	U.S., Colorado	4.1	1.5	2	3-6	0	1	1	1
PI 182932	lanatus	India, Maharashtra	4.1	1.5	2	3-6	0	0	0	0
PI 178872	lanatus	Turkey, Kutahya	4.1	1.1	4	1-7	1	2	1	3
PI 534598	lanatus	Syria	4.1	0.8	7	1-9	4	1	3	2
PI 357682	lanatus	Macedonia	4.1	1	5	1-8.5	2	1	1	1
PI 593376	lanatus	China, Xinjiang	4.2	1.5	2	3-6	0	1	1	0
PI 357660	lanatus	Macedonia	4.2	0.9	6	1-6	2	1	2	2
PI 227204	lanatus	Japan, Aichi	4.2	1.5	2	1.5-7	1	0	0	1
PI 251796	lanatus	Serbia	4.2	1	5	1.5-8	3	1	2	3

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 306366	lanatus	Gabon	4.2	1	5	2-9	2	0	0	1
PI 175651	lanatus	Turkey, Balikesir	4.2	1.5	2	4-4	0	1	0	1
PI 169251	lanatus	Turkey, Mugla	4.2	1.2	3	3-5	0	1	1	2
PI 512377	lanatus	Spain, Alicante	4.2	1.3	3	1.5-7	1	1	2	1
PI 593366	lanatus	China, Xinjiang	4.2	1.1	4	2-7	1	1	2	2
PI 593363	lanatus	China, Xinjiang	4.2	1.1	4	1.7-9	1	2	2	2
PI 222715	lanatus	Iran, Tehran	4.2	1.1	4	1-7	2	1	1	2
PI 635609	lanatus	U.S., California	4.2	1.5	2	1-7	1	0	0	1
PI 368524	lanatus	Montenegro	4.2	1.1	4	2.5-7	0	2	1	1
PI 368512	lanatus	Montenegro	4.2	1.1	4	1.5-6	1	1	1	2
PI 512364	lanatus	Spain, Caceres	4.2	1.5	2	3-4	0	1	1	1
PI 164804	lanatus	India, Maharashtra	4.2	1.5	2	2-5	1	0	0	0
PI 212288	lanatus	Afghanistan, Herat	4.2	1.3	3	3.5-5.2	0	1	0	1
PI 370429	lanatus	Macedonia	4.2	1.1	4	0-7.5	1	0	0	1
PI 635703	lanatus	U.S., Missouri	4.2	1.1	4	1-9	2	0	0	1
PI 593341	lanatus	China, Shanghai	4.2	1.3	3	1-6.5	1	1	0	1
PI 163202	lanatus	India, Punjab	4.2	0.9	7	0-9	3	2	1	3
PI 381706	lanatus	India	4.2	1	5	2.8-6	0	3	1	2
PI 169271	lanatus	Turkey, Kirklareli	4.2	1.3	3	4-5	0	0	0	1
PI 612475	lanatus	NA	4.2	1.3	3	1-7	2	1	0	1
PI 171584	lanatus	Turkey, Tokat	4.2	1.1	4	3-4.5	0	2	2	1
PI 629109	lanatus	U.S., Colorado	4.2	1.1	4	2-6	2	0	1	2
PI 537274	lanatus	Pakistan, Punjab	4.2	1	5	1-8.5	2	2	1	1
PI 172790	lanatus	Turkey, Kars	4.2	0.8	12	1-7	2	2	4	1
PI 635663	lanatus	U.S., California	4.2	1.5	2	1-6	1	0	2	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 277977	lanatus	Turkey, Afyon	4.3	1.1	4	0.8-6	1	1	3	3
PI 357696	lanatus	Macedonia	4.3	0.8	7	2.5-8	0	2	2	3
PI 228342	lanatus	Iran, West Azerbaijan	4.3	1.5	2	3-4	0	1	0	0
PI 593383	lanatus	China, Xinjiang	4.3	1	5	0.5-8	2	1	0	1
PI 214316	lanatus	India, Punjab	4.3	1	5	1-7	2	3	3	2
PI 278055	lanatus	Turkey, Tunceli	4.3	1.3	3	0-6	1	0	1	2
PI 345547	lanatus	Soviet Union, Former	4.3	1.1	4	1-6	1	1	0	1
PI 537465	lanatus	Spain, La Palmas	4.3	1.1	5	1-6	1	1	2	2
PI 593385	lanatus	China, Xinjiang	4.3	1.3	3	1-8	2	0	0	1
PI 500315	lanatus	Zambia	4.3	0.9	6	2-8	1	3	2	1
PI 357704	lanatus	Macedonia	4.3	1.3	3	2-8	1	1	1	1
PI 593379	lanatus	China, Xinjiang	4.3	1	5	0-8	2	1	2	2
PI 173888	lanatus	India, Uttar Pradesh	4.3	1.1	4	2-9	1	0	1	2
PI 482343	lanatus	Zimbabwe	4.3	1.3	3	2-6	1	0	2	0
PI 163204	lanatus	India, Punjab	4.3	1.3	3	3.2-6	0	1	1	1
PI 512343	lanatus	Spain, Zaragoza	4.3	1.3	3	3-6	0	1	1	1
PI 532816	lanatus	China	4.3	1	5	0-9	2	1	1	2
PI 368497	lanatus	Macedonia	4.3	1	5	0.5-8	2	2	2	2
PI 169240	lanatus	Turkey, Antalya	4.3	0.9	7	1-9	3	3	2	3
PI 278011	lanatus	Turkey, Gaziantep	4.4	0.9	6	1-9	1	1	1	2
PI 379232	lanatus	Macedonia	4.4	1.1	4	1.7-9	1	2	2	2
PI 222775	lanatus	Iran	4.4	1.3	3	1-7.5	1	1	1	1
PI 271777	lanatus	South Africa, Transvaal	4.4	1.1	4	1-5.5	1	2	1	2
PI 534588	lanatus	Syria	4.4	1.5	2	2-6	1	0	1	1
C. Gray	lanatus	NA	4.4	0.7	17	0-8	3	2	1	3
PI 381704	lanatus	India	4.4	1	5	2-6	1	2	3	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 173668	lanatus	Turkey, Mus	4.4	1.1	4	3.5-6	0	0	1	3
PI 564535	lanatus	U.S.	4.4	1.5	2	3-7	0	1	1	0
PI 612458	lanatus	Korea, South, Pusan	4.4	1.1	4	1-8	1	1	0	3
PI 179241	lanatus	Iraq	4.4	1.2	3	1-9	2	0	0	1
PI 273481	lanatus	Ethiopia	4.4	1.1	4	3-6	0	1	2	1
PI 169268	lanatus	Turkey, Edirne	4.4	1	9	1-8	1	1	2	2
PI 512404	lanatus	Spain, Cadiz	4.4	1.1	4	1-6	1	1	1	1
PI 593358	lanatus	China, Shaanxi	4.4	1.2	3	1.5-9	2	0	0	1
PI 368505	lanatus	Macedonia	4.4	1	5	2.5-6	0	1	4	0
PI 164550	lanatus	India, Madhya Pradesh	4.4	1.5	2	1-9	1	0	0	1
PI 635659	lanatus	U.S., Minnesota	4.5	1.3	3	2-6	1	2	2	2
PI 385964	lanatus	Kenya	4.5	0.7	14	1-9	4	1	1	1
PI 635606	lanatus	U.S., California	4.5	1.1	4	0-7	1	1	2	1
PI 379222	lanatus	Serbia	4.5	1.3	3	2.5-6.5	0	2	1	1
PI 635715	lanatus	U.S., Michigan	4.5	1.1	4	3-7	0	2	1	2
PI 534599	lanatus	Syria	4.5	1.1	4	1-9	1	1	1	2
PI 500348	lanatus	Zambia	4.5	1.1	4	1-7	1	3	4	2
PI 532810	lanatus	China	4.5	1.3	3	1-9	1	0	0	1
PI 357701	lanatus	Macedonia	4.5	1.1	4	1-9	2	2	1	3
PI 179882	lanatus	India, Gujarat	4.5	1.1	4	1-9	2	0	0	3
PI 314178	lanatus	Uzbekistan, Farghona	4.5	1.1	4	0.5-7	1	2	1	2
PI 185030	lanatus	Turkey, Erzincan	4.5	1.1	4	1-7	1	0	0	0
PI 512347	lanatus	Spain, Tarragona	4.5	1.1	4	0-7	1	1	0	0
PI 270307	lanatus	Philippines	4.5	1.1	4	2.5-8	0	1	1	2
PI 593355	lanatus	China, Shaanxi	4.5	1.5	2	3-5	0	0	0	0

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 164539	lanatus	India, Rajasthan	4.5	1.3	3	0-9	2	0	0	1
PI 278014	lanatus	Turkey, Hatay	4.5	1.1	4	2-7	1	2	2	1
PI 593357	lanatus	China, Shaanxi	4.5	0.9	7	1-7	2	2	1	4
PI 181742	lanatus	Lebanon	4.5	1	5	3.5-5	0	2	3	2
PI 172800	lanatus	Turkey, Urfa	4.6	1.1	4	3-6	0	0	1	1
PI 537271	lanatus	Pakistan, Punjab	4.6	0.9	6	1.5-7	1	3	1	3
PI 482323	lanatus	Zimbabwe	4.6	1.3	3	1-8	1	1	1	2
PI 561122	lanatus	China, Hebei	4.6	1.5	2	4-4.5	0	0	1	1
PI 176495	lanatus	Turkey, Konya	4.6	1.1	4	0.5-9	2	0	0	2
PI 179885	lanatus	India, Gujarat	4.6	1	5	1-7	1	1	0	1
PI 601101	lanatus	U.S.	4.6	1	6	0-9	2	1	2	4
PI 179876	lanatus	India, Rajasthan	4.6	1.2	3	3-6	0	0	0	1
PI 277979	lanatus	Turkey, Afyon	4.6	1.1	4	1-7	1	2	1	1
PI 379251	lanatus	Macedonia	4.6	1.5	2	1-9	1	1	0	1
C. Sweet	lanatus	NA	4.6	0.8	10	1-8	2	1	3	2
PI 278029	lanatus	Turkey, Kirklareli	4.6	1.3	3	3-5.8	0	0	1	1
PI 169286	lanatus	Turkey, Balikesir	4.6	0.9	6	2-9	2	1	2	2
PI 629110	lanatus	U.S., Colorado	4.6	1.3	3	4-5	0	0	0	0
PI 176919	lanatus	Turkey, Manisa	4.6	1.1	4	2.5-9	0	1	1	2
PI 635660	lanatus	U.S., California	4.6	1.3	3	3-8	0	0	2	1
PI 176488	lanatus	Turkey, Erzincan	4.6	0.9	6	1-7	2	4	3	2
PI 560001	mucosospermus	Nigeria, Oyo	4.6	1.1	4	1-9	1	1	0	1
PI 229748	lanatus	Iran, Mazandaran	4.7	1.3	3	0-8	2	1	1	2
PI 169249	lanatus	Turkey, Mugla	4.7	0.9	7	0-9	2	2	2	3
PI 179239	lanatus	Turkey, Tokat	4.7	1.2	3	3-9	0	1	1	2
PI 179234	lanatus	Turkey, Bursa	4.7	1.3	3	0.5-8	1	1	2	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 176913	lanatus	Turkey, Konya	4.7	1.1	4	0-9	1	1	0	1
PI 164977	lanatus	Turkey, Istanbul	4.7	1.2	3	4-5	0	1	1	0
PI 344395	lanatus	Iran	4.7	1.3	3	1-6.5	1	1	2	1
PI 169254	lanatus	Turkey, Izmir	4.7	1.3	4	3.5-8	0	0	1	2
PI 378615	lanatus	Zaire	4.7	1.1	4	0-7	1	2	1	2
PI 608047	lanatus	U.S., Illinois	4.7	1.1	4	1-9	2	1	2	3
PI 250146	lanatus	Pakistan, Punjab	4.7	0.9	8	0.5-9	3	2	1	1
PI 593360	lanatus	China, Shaanxi	4.7	1.3	3	1-5.5	1	1	0	2
PI 176485	lanatus	Turkey, Tunceli	4.7	1.1	4	1-9	1	1	1	1
PI 635726	lanatus	U.S.	4.7	1.1	5	1-8	1	1	1	2
PI 169243	lanatus	Turkey, Antalya	4.7	1	5	0-7	2	0	0	0
PI 482355	amarus	Zimbabwe	4.7	1.1	4	3-8	0	0	1	2
PI 368525	lanatus	Montenegro	4.7	1	5	0-9	2	2	2	1
YB B Dia	lanatus	NA	4.7	0.9	6	0.5-8	2	2	3	5
PI 172789	lanatus	Turkey, Kars	4.8	1.5	2	3-6	0	0	1	0
PI 192938	lanatus	China, Shanghai	4.8	1.1	4	3-7	0	2	1	1
PI 169281	lanatus	Turkey	4.8	1.3	3	3-7	0	0	0	1
PI 482371	lanatus	Zimbabwe	4.8	1	5	0.5-8	2	2	2	3
PI 270145	lanatus	Greece	4.8	1.2	3	0-7	1	0	0	0
PI 525090	lanatus	Egypt, Asyut	4.8	1	5	1.5-9	2	1	1	4
PI 169238	lanatus	Turkey, Manisa	4.8	1.5	2	1-7	1	0	0	0
PI 176489	lanatus	Turkey, Sivas	4.8	0.9	6	1-7	1	1	1	4
PI 181938	lanatus	Syria	4.8	1.1	4	2-6.2	1	1	0	1
PI 381731	lanatus	India	4.8	1.1	4	2-7	1	0	0	2
PI 505589	lanatus	Zambia	4.8	1.5	2	4-5.5	0	0	0	1
PI 192937	lanatus	China, Shanghai	4.8	1.3	3	3-7	0	1	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 368495	lanatus	Macedonia	4.8	1	5	2.7-5	0	1	1	4
PI 470247	lanatus	Indonesia	4.8	1.1	4	2-7	1	2	0	2
PI 379257	lanatus	Montenegro	4.8	1	5	0.5-8	1	1	1	3
PI 274795	lanatus	Pakistan	4.8	1.2	3	0-9	1	0	1	2
PI 181743	lanatus	Lebanon	4.8	1.3	3	2-9	1	1	0	1
PI 183124	lanatus	India, Gujarat	4.8	1.3	3	1-6	1	1	2	2
PI 271751	lanatus	Ghana, Upper	4.8	1.3	3	4-6	0	0	0	0
PI 482351	lanatus	Zimbabwe	4.9	0.9	7	2-8	2	1	2	0
PI 290855	lanatus	U.S.	4.9	1.3	3	5-5	0	0	1	2
PI 174104	lanatus	NA	4.9	1.5	2	4.5-5.5	0	0	0	1
PI 357721	lanatus	Macedonia	4.9	1.1	4	2-9	1	1	1	1
PI 357724	lanatus	Macedonia	4.9	1.2	3	2-8.5	2	1	2	1
PI 368496	lanatus	Macedonia	4.9	1.1	4	0-8	2	2	1	2
PI 345544	lanatus	Ukraine	4.9	1	5	2-8	1	2	3	1
PI 441722	lanatus	Brazil, Federal District	4.9	1.1	4	3-6	0	2	2	2
PI 226634	lanatus	Iran, Kerman	4.9	1.5	2	3.5-6	0	1	1	1
PI 379241	lanatus	Macedonia	4.9	1.3	3	1-9	1	1	0	1
PI 432337	colocynthis	Cyprus	4.9	1.3	3	3.7-6	0	1	3	2
PI 635618	lanatus	U.S., California	4.9	1.3	3	2.5-8	0	1	0	1
PI 182933	lanatus	India, Maharashtra	4.9	1	5	3-9	0	0	0	2
PI 169233	lanatus	Turkey, Manisa	4.9	1.5	2	4-6	0	0	0	0
PI 175102	lanatus	India, Uttar Pradesh	4.9	1	5	1.7-8	1	2	2	2
PI 266028	lanatus	Venezuela, Aragua	5	1.3	4	3-9	0	0	2	3
PI 538888	lanatus	Russian Federation	5	1.3	3	3.2-6	0	1	0	2
PI 635683	lanatus	U.S., Wyoming	5	1	5	2.5-8	0	0	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 601289	lanatus	U.S.	5	1.5	2	5-6	0	1	0	1
PI 442826	lanatus	Brazil, Mato Grosso	5	1	5	2.5-7	0	2	2	3
PI 525087	lanatus	Egypt, Qena	5	1.3	3	2-8.5	1	1	0	2
PI 277989	lanatus	Turkey, Aydin	5	1.1	4	0-7	1	2	0	2
PI 270550	lanatus	Ghana	5	1.1	4	3-5.5	0	2	2	1
PI 381696	lanatus	India	5	0.9	6	3-9	0	1	0	2
PI 179883	lanatus	India, Gujarat	5	1.1	4	3.5-9	0	0	0	2
PI 226445	lanatus	Israel, Tel Aviv	5	1.3	3	2-7	1	2	0	2
PI 357725	lanatus	Macedonia	5	1.1	4	3-8	0	1	2	2
PI 635668	lanatus	U.S., Wyoming	5	0.8	13	1.5-8	3	4	1	7
PI 512350	lanatus	NA	5.1	1.5	2	2-8	1	0	0	1
PI 635611	lanatus	NA	5.1	1.5	2	3-7	0	0	1	1
PI 629108	lanatus	U.S., California	5.1	0.7	17	0-8	4	3	1	5
Fascination	lanatus	NA	5.1	1.5	2	4-7	0	1	2	0
PI 169263	lanatus	NA	5.1	1.5	2	4.5-6	0	0	1	1
PI 176922	lanatus	Turkey, Manisa	5.1	1	5	1-8	1	3	2	1
PI 357700	lanatus	Macedonia	5.1	1.3	3	3-9	0	0	0	2
PI 212287	lanatus	Afghanistan, Herat	5.2	1.2	3	3.5-7	0	2	1	2
PI 368501	lanatus	Macedonia	5.2	1.1	4	1-7	1	0	1	1
PI 635635	lanatus	U.S., South Carolina	5.2	1.5	2	2-9	1	0	0	1
PI 635728	lanatus	U.S.	5.2	1.3	3	1-9	1	0	0	2
PI 164247	lanatus	Liberia	5.2	1.1	4	1-9	2	1	1	2
PI 357673	lanatus	Macedonia	5.2	1.1	4	2-7	1	3	2	2
PI 182934	lanatus	NA	5.2	1.5	2	4-6	0	1	0	1
PI 632753	lanatus	U.S., Maryland	5.2	1.3	3	2-7	1	0	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 543210	lanatus	Bolivia, Beni	5.2	1.2	3	4-6.5	0	1	2	1
PI 174099	lanatus	Turkey, Elazig	5.2	0.8	7	1-9	2	3	4	3
PI 368522	lanatus	Macedonia	5.3	1.3	3	3-9	0	1	1	2
PI 211849	lanatus	Iran, Tehran	5.3	1.1	4	3-9	0	1	3	2
PI 249559	lanatus	Thailand	5.3	1	5	3-9	0	1	0	2
PI 176905	lanatus	Turkey, Edirne	5.3	1.3	3	3-7.2	0	1	1	2
PI 612464	lanatus	Korea, South, Pusan	5.3	1.1	4	2-7	1	1	2	1
PI 593352	lanatus	China, Henan	5.3	1.1	4	2-8	1	1	0	2
PI 164146	lanatus	India	5.3	1.3	3	3-9	0	1	1	2
PI 381722	lanatus	India	5.3	1.5	2	4.5-6.3	0	1	2	1
PI 278023	lanatus	Turkey, Kars	5.3	1	5	1-9	2	0	0	3
PI 435085	lanatus	China, Xinjiang	5.3	1.5	2	3-8	0	1	1	1
PI 537273	lanatus	Pakistan, Punjab	5.4	0.9	6	2.5-9	0	2	2	3
PI 431579	lanatus	India	5.4	1.3	3	2-8	1	0	1	2
PI 169235	lanatus	Turkey, Manisa	5.4	1.5	2	4.5-6	0	0	0	1
PI 458739	lanatus	Paraguay, Chaco	5.4	1.2	3	3-8	0	2	1	2
PI 512396	lanatus	Spain, Valencia	5.4	1.1	4	3-9	0	0	0	1
PI 222712	lanatus	Iran, West Azerbaijan	5.4	1.2	3	4-7	0	1	0	3
PI 537269	lanatus	Pakistan, Punjab	5.4	1	5	3-7	0	1	0	1
PI 219907	lanatus	Afghanistan	5.4	1.5	2	4-7	0	1	2	1
PI 512374	lanatus	Spain, Alicante	5.4	1.3	3	1-9	1	1	1	1
PI 525082	colocynthis	Egypt	5.5	1.2	3	0.5-9	1	0	1	1
PI 512378	lanatus	Spain, Valencia	5.5	1.3	3	1-9	1	0	0	1
PI 379242	lanatus	Macedonia	5.5	1.5	2	1-9	1	0	0	2
PI 169277	lanatus	Turkey, Canakkale	5.5	1.1	4	2-9	1	0	1	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 368515	lanatus	Macedonia	5.5	1.1	4	3-7	0	2	3	0
B Diamond	lanatus	NA	5.5	1.5	2	5-5.8	0	1	1	2
PI 635664	lanatus	U.S., California	5.5	1.3	3	3-6	0	1	0	0
PI 593368	lanatus	China, Xinjiang	5.5	1.3	3	3.4-7	0	1	0	2
PI 534591	lanatus	Syria	5.5	1.5	2	3-7	0	2	0	1
PI 370424	lanatus	Macedonia	5.5	1.1	5	4.3-9	0	1	1	2
PI 635600	lanatus	U.S., California	5.5	1.3	3	5-5.2	0	0	0	1
PI 279460	lanatus	Japan	5.5	1.5	2	1-9	1	0	0	1
PI 164655	lanatus	India, Karnataka	5.5	1.3	3	4-8	0	2	1	1
PI 593350	lanatus	China, Henan	5.6	1.1	4	4-9	0	0	0	1
PI 180277	lanatus	NA	5.6	1.3	3	3-8	0	0	0	0
PI 212596	lanatus	Afghanistan	5.6	1.5	2	4.3-6	0	0	1	1
PI 512405	lanatus	Spain, Cadiz	5.6	1.2	3	4-7	0	0	0	0
PI 357687	lanatus	Macedonia	5.6	1.5	2	5-6	0	1	1	0
PI 357723	lanatus	Macedonia	5.6	1.3	3	1.5-9	1	0	1	1
PI 179242	lanatus	Iraq	5.6	1.1	4	4-9	0	1	1	3
PI 635626	lanatus	U.S., California	5.6	1.5	2	4-7	0	1	1	1
PI 254430	lanatus	Lebanon	5.6	1.1	4	2.5-8	0	1	2	3
PI 270306	lanatus	Philippines	5.6	1.1	4	1-9	1	1	0	2
PI 278053	lanatus	Turkey, Tunceli	5.6	1.1	4	3-8	0	3	1	3
PI 271984	lanatus	Somalia	5.6	0.9	6	1.5-9	2	2	3	3
PI 357753	lanatus	Macedonia	5.6	1.5	2	5-7	0	2	0	2
PI 635661	lanatus	U.S., California	5.8	1.3	3	5-6	0	1	2	2
PI 278058	lanatus	Turkey, Usak	5.8	1.1	4	1-9	1	2	2	3
PI 172794	lanatus	Turkey, Van	5.8	1.3	3	2.5-9	0	0	1	2
PI 537471	lanatus	Spain, Murcia	5.8	1.5	2	2-9	1	0	0	1

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 278005	lanatus	Turkey, Canakkale	5.8	1.3	3	4.5-8	0	1	0	3
PI 381725	lanatus	India	5.8	1.5	2	4-8	0	2	1	1
PI 635594	lanatus	U.S., California	5.8	1.5	2	3.7-8	0	1	1	1
PI 211851	lanatus	Iran, Tehran	5.8	1.1	4	3.5-7	0	2	2	2
PI 271981	lanatus	Somalia	5.8	1.3	3	3-9	0	1	1	2
PI 357742	lanatus	Macedonia	5.9	1.5	2	6-6	0	0	0	0
PI 226506	lanatus	Iran, Khuzestan	5.9	1.5	2	3.5-9	0	0	0	1
PI 172791	lanatus	Turkey, Kars	5.9	1.5	2	0-9	1	0	0	1
PI 438673	lanatus	Mexico, Yucatan	5.9	1.2	3	4-9	0	1	1	2
PI 435990	lanatus	China, Shaanxi	5.9	1.5	2	4-8	0	1	1	0
PI 662034	lanatus	Turkmenistan	5.9	1.1	4	2-7	1	2	1	1
PI 593370	lanatus	China, Xinjiang	5.9	1.5	2	1.5-8	1	0	1	1
PI 534592	lanatus	Syria	6	1.3	3	3-9	0	2	2	3
PI 269679	lanatus	Belize	6	1.5	2	3-6.5	0	1	1	1
PI 165523	lanatus	India	6	1.1	4	1-9	1	0	0	2
PI 612468	lanatus	Korea, South, Pusan	6	1.1	4	4-9	0	1	0	2
PI 271982	lanatus	Somalia	6	1.2	3	2-9	1	0	1	2
PI 195771	lanatus	Guatemala, Izabal	6	1.1	4	5.2-8	0	2	2	3
PI 547106	lanatus	U.S.	6.1	1.2	3	3-8	0	1	0	2
PI 175652	lanatus	Turkey, Kastamonu	6.1	1.1	4	3-9	0	0	0	3
PI 163205	lanatus	India, Punjab	6.1	1.3	3	3-9	0	1	1	2
PI 220778	colocynthis	Afghanistan, Farah	6.1	1.5	2	3-8	0	2	1	0
PI 379227	lanatus	Macedonia	6.1	1.2	3	1-9	1	0	0	3
PI 368526	lanatus	Montenegro	6.1	1.2	3	4-7	0	0	2	2
PI 278042	lanatus	Turkey, Mugla	6.2	1.3	3	2-9	1	1	1	1
PI 507868	lanatus	Hungary, Szabolcs-Szatmar	6.2	1.1	4	2-9	1	1	0	2

Appendix B. Continued.

Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Symptom Incidence[v]		
								Blotching	Cracking	Necrosis
PI 169250	lanatus	NA	6.2	1.5	2	2.5-9	0	0	0	1
PI 197416	lanatus	Ethiopia	6.2	1.1	4	4-8	0	3	2	2
PI 536450	lanatus	Maldives	6.2	1.2	3	5-9	0	0	1	1
PI 179238	lanatus	Turkey, Canakkale	6.3	1.5	2	3.7-9	0	0	0	1
PI 635721	lanatus	U.S., Maryland	6.3	1.1	4	0-9	1	0	0	2
PI 534593	lanatus	Syria	6.4	1.5	2	2-9	1	0	0	2
PI 234605	lanatus	Singapore	6.4	1.3	3	3.5-8.5	0	1	1	2
PI 179886	lanatus	India, Gujarat	6.4	1.3	3	5-9	0	0	1	2
PI 601221	lanatus	U.S., Florida	6.5	1.5	2	5.5-7	0	1	1	2
PI 277976	lanatus	Turkey, Adiyaman	6.5	1.1	4	2.5-9	0	1	0	2
PI 211852	lanatus	Iran, Tehran	6.5	1.3	3	5-9	0	0	0	1
PI 172803	lanatus	Turkey, Maras	6.5	1.2	3	2.2-9	0	1	2	1
PI 388021	lanatus	India, Karnataka	6.5	1.3	3	6-7	0	2	1	1
PI 254716	lanatus	Sudan, Khartoum	6.5	1.3	3	4.5-9	0	2	0	3
PI 357736	lanatus	Macedonia	6.5	1.3	3	2-9	1	1	0	1
PI 278017	lanatus	Turkey, Izmir	6.6	1.5	2	2.5-9	0	0	0	1
PI 507860	lanatus	Hungary, Szabolcs-Szatmar	6.7	1	5	5-9	0	1	0	3
PI 381734	lanatus	India	6.7	1.2	3	4-9	0	0	1	2
PI 368498	lanatus	Macedonia	6.8	1.2	3	4-9	0	0	0	1
PI 368507	lanatus	Macedonia	6.9	1.3	3	4-9	0	1	1	2
Cobb-Gem	lanatus	NA	6.9	1.5	2	5-9	0	0	0	1
PI 164992	lanatus	Turkey, Ankara	7	1.1	4	5-8	0	1	1	3
PI 435282	lanatus	Iraq	7.2	1.2	3	5-9	0	0	0	2
PI 274785	lanatus	India, Delhi	7.2	1.2	3	1.5-9	1	1	1	2
PI 525091	lanatus	Egypt, Asyut	7.4	1	5	3.3-9	0	2	1	4

Appendix B. Continued.

								Symptom Incidence[v]		
Cultigen	Taxonomy[z]	Origin[z]	Rating[y]	S.E.[y]	No.[x]	Range	No. ≤ 2[w]	Blotching	Cultigen	Necrosis
PI 270545	lanatus	Sudan, Khartoum	7.5	1	5	4-8	0	3	1	4
PI 169247	lanatus	Turkey, Mugla	7.5	1.5	2	5.7-9	0	0	0	1
PI 635672	lanatus	U.S., Wyoming	7.7	1.3	3	5-9	0	2	2	2
PI 169246	lanatus	Turkey, Mugla	7.7	1.5	2	4-9	0	0	0	1
PI 357746	lanatus	Montenegro	7.7	1.5	2	5-9	0	0	0	1
PI 525084	lanatus	Egypt, Qena	8.4	1.5	2	8-9	0	1	0	1
PI 370423	lanatus	Macedonia	8.5	1.3	3	6-9	0	0	1	3
PI 222184	lanatus	Afghanistan, Kandahar	9	1.5	2	8-9	0	0	0	2
PI 164748	lanatus	India, Karnataka	9.7	1.3	3	9-9	0	0	1	2
Standard Error of the Difference										
Minimum	0.8									
Average	1.6									
Maximum	2.2									

[z]According to the late Nov 2017 Germplasm Resource Information Network (GRIN) web server (https://www.ars-grin.gov/).
[y]Ratings and standard errors represent disease severity BLUEs calculated ASREML-R 3.0 (Butler et al., 2009) on lines with a minimum of two observations.
[x]The total number of observations for each cultigen over 2015-2017.
[w]The number of observations with a rating ≤ 2.
[v]The number of fruit exhibiting any noticeable degree of symptoms other than blistering. Fruit generally exhibited a combination of symptoms. Blotching refers to fruit surface discoloration; cracking indicates that the outer surface was broken; and necrosis means that the infection had caused internal necrosis.